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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States
Walter Wyman, Surgeon-General

ABORATORY.—BULLETIN No. 59-62
December, 1909

THE OXIDASES

AND OTHER OXYGEN-CATALYSTS CONCERNED IN BIOLOGICAL OXIDATIONS

By

J. H. KASTLE

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THE OXIDASES AND OTHER OXYGEN-CATALYSTS CONCERNED IN BIOLOGICAL OXIDATIONS.[a]

By Joseph H. Kastle,

Chief, Division of Chemistry, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

INTRODUCTION.

During the past eight years I have devoted a considerable part of my time to a study of the oxidases and of certain phases of biological oxidation, with the result that I have come to appreciate as never before the need of a somewhat more comprehensive and complete treatment of this subject than is ordinarily to be found in most treatises on fermentation. Furthermore, a study of the earlier literature of the subject has revealed certain rather curious mistakes, which probably found their way into the literature originally as the result of typographical errors, but which pervade the whole subject with an annoying persistency. During the past four years, as the result of easy access to the departmental libraries in this city, I have had unusual facilities for familiarizing myself with the literature of this subject, and it was partly with the view of giving to other workers in this field the benefits of my opportunities along this line, that this monograph has been written. No special claims are made for originality either in the mode of treatment of the subject, or in some cases even with regard to phraseology, my only object being to present the subject as accurately and comprehensively as possible within the time at my disposal. To this end I have made free use of such treatises on fermentation as "The soluble ferments and fermentation," by J. Reynolds Green (Cambridge Natural Science Manuals, London, 1899); "Die Fermente und ihre Wirkungen," by Carl Oppenheimer, (Leipzig, 1900); "Traite de Microbiologie," by E. Duclaux (Paris, 1898); "Biochemie der Pflanzen," by Fred. Czapek (Jena, 1905); "Theorie der Fermentwirkungen," by Moritz Traube (Berlin, 1858); "Les Enzymes et leurs Applications," by Effront (translation by Prescott, New York, 1902); "Recent advances in physiology and biochemistry," by Leonard Hill and others (London, 1906); "Das Sauerstoff-Bedürfniss des Organismus," by Paul Ehrlich (Berlin, 1885); "Les Oxydations de l'Organisme," by E. Enriquez and J.-A. Sicard (Paris, 1902); "Die Bedeutung der Katalyse für die Medicin," by H. Schade (Kiel, 1907); and "Kritische Studien über die Vorgänge der Autoxydation," by C. Engler and J. Weissberg (Bräunschweig, 1904). I am especially indebted for much valuable information to a dissertation by Pierre Sée, entitled "Contribution a l'Etude des Applications Therapeutiques des Oxydases et des Metaux Ferments" (Thèse, No. 239, Faculté de Médicine de Paris, 1905), and to recent articles by Engler and Herzog, "Zur chemischen Erkenntnis biologischer Oxydationsreaktionen" (Hoppe-Seyler's Zeitschrift für physiologische Chemie, 1909, vol. 29, pp. 327–375), and to Moore and Whitley, "The properties and classification of the oxidizing enzymes and analogies between enzymic activity and the effects of immune bodies and complements" (Bio-Chemical Journal, 1909, vol. 4, pp. 136–167). In many instances in dealing with original contributions to the subject I have adhered as closely as possible to the words of the author in order that the exact sense and meaning of the original article might be preserved to at least as great an extent as possible within the limits of the present communication. Furthermore, no claim is made for the completeness of this production. It has been impossible, through lack of time and space, to refer to all original communications on the subject, even in the bibliography. Even the current numbers of the journals contain many contributions bearing on the subjects treated of in the following pages, and out of this vast and constantly growing literature I have been compelled to select what seemed to me the most essential to the proper presentation of the subject.

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[a] Manuscript submitted for publication October 12, 1909.

In the writer's opinion the subjects considered in the following pages are strictly germane in their general scope to the work of the Hygienic Laboratory. The oxidases and related oxygen-catalysts belong to the same group of bodies as the serums, viruses, toxins, and antitoxins. Any facts throwing light on the one class of substances will doubtless assist ultimately to a better understanding of this whole group of biologically active substances. Portier is of the opinion that the oxidases play an important rôle in the defense of the organism against pathogenic micro-organisms, and according to Sieber they are able to destroy toxins, such as the toxins of diphtheria and tetanus. Furthermore, they are of importance as enabling us to form correct conclusions regarding the condition and character of certain foodstuffs—as to whether they are cooked or raw—and in enabling us to determine, within certain limits of temperature, whether a given sample of milk has been pasteurized or not. For the biological chemist, the physician, and the sanitarian, all of these matters are of considerable importance.

I am greatly indebted for much valuable material to various authors, whose works I have freely consulted and made use of in the preparation of this monograph, and also to Mr. F. A. McDermott, one of the assistants in the division of chemistry of the Hygienic Laboratory, for much valuable assistance.

Chapter I.

THEORIES OF OXIDATION.

In the period which elapsed between the classic researches of Lavoisier on Combustion (1774–1785) and the discovery of ozone by Schoenbein in 1840, a number of interesting and important observations had accumulated in the literature concerning the influence exerted by one substance on the oxidation of another by air or oxygen. Thus in 1806 Désormes and Clement ([137]) pointed out that nitric acid is not the principal agent concerned in the complete oxidation of the sulfur in the leaden-chamber process for the manufacture of sulfuric acid, but its "base," nitric oxide (*le gaz nitreux*), which takes oxygen from atmospheric air in order to offer it to the sulfurous acid in a condition capable of accomplishing its oxidation. A few years later it was observed by Vogel ([443]) that hydrogen and oxygen combine at low temperatures under the influence of charcoal. Then followed the numerous and interesting observations by Sir Humphrey Davy ([134]), Edmund Davy ([132–133]), Erman ([167]), Pleischl ([328]), and others on the remarkable power of platinum and other metals of the platinum group, such as iridium, osmium, and palladium, to effect the slow combustion, or under certain conditions even the actual ignition, of combustible gases such as hydrogen, carbon monoxide, etc., in an atmosphere containing oxygen, and the rapid conversion of alcohol into acetic acid, observations which culminated in Davy's lamp without flame (*see* Erman [167]), Doebereiner's lamp ([141]), and the rapid method for the production of acetic acid.

The effect of various substances on the oxidation of combustible gases and vapors was also exhaustively investigated by Dulong and Thenard ([151–152]), with the result that various metals, such as iridium, palladium, rhodium, gold, silver, mercury, nickel, cobalt, and iron, in spongy or pulverulent form, were found to accomplish the same changes as platinum, and that to a degree at least such changes can also be brought about by certain nonmetallic substances, such as carbon, pumice stone, porcelain, glass, and quartz crystals, at temperatures under 350° C., while salt, fluorspar, and marble did not appear to act to a sensible extent within these limits. These changes, they concluded, could not be ascribed entirely to electrical phenomena, as was at first supposed, and they explained them on the assumption of the condensation of large volumes of gas on the surface of the substance capable of causing the oxidation—as, for example, the condensation of oxygen upon platinum, whereby heat was liberated and the body (platinum) attained the temperature necessary to accomplish the oxidation.

The discovery of ozone by Schoenbein (367) in 1840 and the finding of this remarkable substance among the oxidation products of readily oxidizable substances gave a new impulse to the study of oxidation phenomena and led ultimately to a more careful study of the whole subject of induced oxidations and of the phenomenon of oxygen carrying. This distinguished observer seems to have been the first to clearly recognize that in the final products of chemical combination, particularly those in which oxygen is concerned, we see, as he put it, only the closing scene of a chemical drama which is in reality composed of several intermediate acts, and that for a correct understanding of such processes it is quite as essential to know these intermediate acts as it is to know the beginning and the end.

To the investigation of ozone and to the task of learning more of the initial and intermediate acts of the oxygen drama, Schoenbein set himself with remarkable assiduity and success. It is beyond the scope of this communication to attempt anything like a chronological account of the numerous investigations on the subject of ozone, which in themselves form one of the most interesting chapters in the history of chemical science. (*See* Engler and Weissberg[164].) It is sufficient to say in this connection that from the time of its discovery until its composition and nature were finally settled through the labors of Odling, Soret, and Andrews and Tait, Schoebein himself held different notions at different times respecting its composition. As the net result of his many researches on the subject, Schoenbein came to look upon ozone and the ozonides as containing an atom of negatively polarized oxygen, which in its conduct toward oxidizable substances was far more active than oxygen in its ordinary form, and as we would interpret these phenomena at the present day, he accounted for its production from ordinary oxygen through the action of electricity upon the supposition that as a result of the electrical discharge the ultimate particle (molecule) of oxygen is resolved into two atoms, one of which is negatively polarized and the other positively polarized, thus:

$$(O) = \oplus + O \text{ or, } O_2 = \oplus + \ominus$$

The negatively polarized atom them combines with a particle of ordinary oxygen to form ozone, $O_2\ominus$, whereas the positively polarized atom (antozone) combines with water to form hydrogen peroxide, $H_2O.\oplus$. These two substances, ozone and hydrogen peroxide, were regarded as the prototypes of a whole series of compounds to which Schoenbein gave the name ozonides and antozonides, respectively.

According to Hagenbach (203), Schoenbein did not hold that the oxygen molecule consisted of two atoms. Therefore, according to Schoenbein, the chemical polarization of neutral oxygen is no true decomposition, but only a calling into being of two oppositely active states of the element—

$$(O) = \oplus + \ominus$$

The $\oplus$ combines with water to form H_2O_2, and the $\ominus$ goes partly to form ozone, but in greatest part to oxidize the metal or phosphorus.

Like ozone, the ozonides were believed to contain a part of their oxygen in a negatively polarized condition, whereas the antozonides were supposed to contain a part of their oxygen in a positively polarized condition. Like ozone itself, the ozonides were supposed to contain a part of their oxygen in an intensely active condition. On the other hand, in antozone and the antozonides the antozonic oxygen was supposed to be less active chemically than the active oxygen in ozone and the ozonides, at least in most of its chemical relations, and to exhibit chemical activities of a different order. The following is a list of some of the common ozonides and antozonides included in this classification:

$O_2.\ominus$, ozone.
$PbO.\ominus$, lead peroxide.
$MnO.\ominus$, manganese peroxide.
$HCl.\ominus$, hypochlorous acid.
etc.

$\oplus$, antozone.
$H_2O.\oplus$, hydrogen peroxide.
$BaO.\oplus$, barium peroxide.
etc.

This view regarding the nature of ozone and antozone was apparently in harmony with a great many facts, among which may be mentioned:

1. Modes of formation from ordinary oxygen by the action of electricity and as a result of the autoxidation of readily oxidizable substances such as phosphorus, its production in the electrolysis of water and as the result of heating various oxides and peroxides and in the decomposition of highly oxygenated compounds by acids.

2. The greater oxidizing power of ozone and the ozonides as compared with the oxidizing power of antozone and the antozonides. Thus it was proven by Schoenbein that ozone and the ozonides can accomplish the oxidation of a number of metals, guaiacum, etc., which are not acted upon by hydrogen peroxide or other antozonides.

3. The mutual decomposition of ozone and the ozonides by antozonides resulting in the formation of ordinary oxygen. Such decomsitions are those met with in the decomposition of hydrogen peroxide by lead and manganese dioxides and by potassium permanganate, and also the remarkable decomposition of ozone itself by hydrogen peroxide, whereby water and ordinary oxygen are formed.

4. The apparent occurrence of antozone in nature in certain varieties of fluorspar, and its apparent formation (or the actual formation of hydrogen peroxide, an antozonide) by the action of an acid on an antozonide like barium peroxide.

While the differences existing between the so-called ozonides and antozonides and also their mutual decomposition, can be explained upon grounds[a] other than those involving the idea of oppositely electrified oxygen atoms, Schoenbein's theories respecting the nature of this interesting group of substances were more or less in harmony with the dualistic theory of Berzelius and with the fact that the molecule of oxygen consists of two atoms. Their principal interest in this connection is that erroneous as they were in some particulars, they afforded a satisfactory explanation of oxygen activation. A few examples will serve to make this clear: When a solution of indigo blue is exposed to oxygen or atmospheric air it suffers no change. On the other hand, as is well known, phosphorus is readily oxidized under these conditions. And now, what is more remarkable, if indigo be brought in contact with slowly oxidizing phosphorus, not only does the phosphorus continue to be oxidized but under these new conditions the indigo is oxidized as well. In other words, oxidizing phosphorus has the power to excite or induce the oxidation of a substance which alone is incapable of undergoing oxidation by simple contact with oxygen or atmospheric air. As shown by Schoenbein, however, when phosphorus is slowly oxidized in the air ozone is produced and it is the latter substance which oxidizes the indigo. When a solution of potassium iodide and starch or a suspension of guaiacum resin in water is shaken with air, no change of color occurs. If, however, as shown by Schoenbein (380), a drop or two of oil of bitter almonds be added to these solutions and the solutions afterwards shaken with air, each of them becomes blue in color, due in the first case to the oxidation of the potassium iodide with the setting free of iodine and the formation of the blue iodide of starch, and in the second case to the oxidation of the guaiacum with the production of guaiacum blue. According to Schoenbein, the oil of bitter almonds ozonized the air and the resulting ozone oxidized these substances which are not oxidizable by oxygen in its ordinary form.

He also made the interesting observation that certain of the higher fungi, as well as other plants, contain substances which apparently have the power of ozonizing the air to a remarkable degree, thereby accomplishing the oxidation of substances not oxidizable by ordinary oxygen, and in certain instances resulting in the development of characteristic vegetable colors (378). He proved, further, that just as a large number of substances have the power of activating the oxygen of the air, so also an even larger number have the power of activating the oxygen of hydrogen peroxide and other antozonides. Thus while hydrogen peroxide and old oil of turpentine are without action on guaiacum or a solution of potassium iodide and starch, the oxidation and bluing of these reagents are readily accomplished by these oxidizing agents through the action of platinum black, extract of malt, the red coloring matter of the blood, and the juices and extracts of many plants, for the reason that, according to Schoenbein (383), they are converted into ozonides, in the same way that an ozonide, like lead peroxide, results from the action of lead acetate on hydrogen peroxide.

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[a] Mendelejeff ([illegible]) divides the peroxides into two classes, 1, superoxides, in which the oxygen atoms are directly united to one another as well as to the oxidized element, and 2, polyoxides, in which the oxygen atoms are not directly united with one another. The superoxides yield hydrogen peroxide when dissolved in acids; the polyoxides do not. Similar views are held by Traube and by Richarz ([illegible]).

He also proved that ozone is formed during many processes of combustion, an observation which led to the conclusion that every combustion is accompanied by the formation of ozone, and finally to the view that no oxidation can proceed of itself without the previous conversion of the common inactive oxygen of the air into active oxygen or ozone. In this manner he readily accounted for the phenomenon of oxygen carrying, and arrived at the further conclusion that through the agency of living things and the organic matter on the surface of the earth, the inactive oxygen of the air is constantly being transformed into ozone. This gradual formation of ozone through these agencies was deemed sufficient to account for the slow oxidations continually taking place on the surface of the earth, and in speaking of the oxygen-carrying power of certain substances contained in the blood of animals, which like phosphorus and oil of bitter almonds can activate the inactive respired oxygen, he says that "as a matter of fact, without the presence of such substances as convert ordinary oxygen into ozone, animals would be suffocated in the midst of an ocean of the purest but inactive oxygen as quickly as in a vacuous space." (Schoenbein (380).)

Indeed, upon every hand, apparently, facts and observations multiplied, indicating the importance of ozone in the economy of nature. Hare (205) observed its production as the result of rubbing a piece of flint. For example, the odor of ozone is very evident when flint is struck with a piece of steel. Brame (93) detected ozone in rain water. Scoutetten (394, 395, 396, 397) observed its production in the evaporation of impure waters. As a matter of fact ozone became the chemical fad of the day, with the result that its importance in nature was greatly overestimated. Thus during the years 1866–67 daily observations were made on the amount of ozone in the atmosphere of Paris and other localities, and everywhere men were busily engaged in studying its relation to health and disease. Its absence from the atmosphere of certain localities, or its presence therein in diminished amounts was associated with several violent outbreaks of Asiatic cholera that occurred during this period, and according to Doctor Moffatt (303), an English physician, the approach of an ozone period, during which the quantity of ozone in the atmosphere suffers a considerable increase, is followed by a corresponding increase in the luminosity of the glowworm and of certain phosphorescent protozoa, and even of phosphorus itself, when exposed to the air; and in man by a decided increase in the output of phosphates in the urine. Further, according to Doctor Moffatt, the advent of an ozone period was found to be marked by the approach of thunderstorms and unsettled weather conditions generally, and by a marked increase in the number of cases of toothache, neuralgia, apoplexy, etc. Finally, as the result of a large number of observations extending over a period of ten years, the most exact agreement was established between the warnings of the admiralty cautionary telegrams, as the British weather forecasts were then called, and the readings of the Doctor's ozonometer. The Doctor himself had evidently fallen a victim to ozone.

Despite, however, the great number of faulty and imperfectly controlled observations on this remarkable substance and the erroneous theories arising therefrom, the fact remains that this remarkably active form of oxygen is produced under a great variety of conditions, and that it is responsible for the oxidation of many substances not ordinarily oxidized by atmospheric oxygen (*see* Wurster (464)); and the fact that it is formed as a by-product in certain processes of autoxidation affords a simple explanation of oxygen carrying on the part of such substances as give rise to ozone during autoxidation.

From the ozone theory of Schoenbein as a point of departure, our theoretical conceptions regarding processes of autoxidation and the phenomenon of oxygen-carrying have been developed mainly along three different lines. Briefly stated these conceptions are: (1) The ionization theory of Van 't Hoff; (2) the theory of Hoppe-Seyler; and (3) the peroxide theory of Traube, Engler, Bach, and Manchot.

Van 't Hoff's theory of oxygen-activation rests upon the assumption that in slow oxidation, such as that met with in the gradual oxidation of phosphorus, the oxidizable substance enters into combination, not with molecular oxygen, but with the very small amounts of atomic (ionic) oxygen which are constantly being produced from molecular oxygen in the sense of the equilibrium $O_2 = 2O$, and that in a gaseous system such as that furnished by acetic aldehyde and gaseous oxygen, the velocity of the reaction should be exactly proportional to the square root of the oxygen pressure.

The idea that molecular oxygen dissociates as the result of electrification and under the influence of heat and chemical action, is by no means a new one, nor did it originate with Van 't Hoff. As a matter of fact, it had been promulgated by Clausius ([119, 120, 121]) upon theoretical grounds and as affording a simple explanation of the differences between active and common oxygen, as early as 1857 to 1863, and has since been employed by other physicists (among them Schuster, Von Helmholtz, Richarz, Thomson, and others), as affording a simple explanation of the phenomena observed in the electrification of gases, including oxygen, and in the condensation of aqueous vapor in the steam jet as the result of the oxidation of various substances in close proximity thereto. Thus Clausius ([119]) in a communication entitled "Ueber die Art der Bewegung, welche wir Wärme nennen," had put forward the suggestion that in common oxygen the atoms are not detached, but combined in twos to form molecules, a conclusion which also derived support from the views of Gerhardt on the constitution of gaseous molecules. He reached the conclusion therefore that the active oxygen which common oxygen sometimes contains and which at this time was not distinguished from ozone, consists not of atoms combined in pairs, but of single atoms distributed among the molecules of the element in its ordinary form, and in a later communication on the difference between active and common oxygen ([121]) this author reiterates the view that common oxygen consists of paired atoms, and active oxygen of unpaired atoms, and further that the two atoms which go to form ordinary oxygen are in oppositely electrified states. Hence, according to Clausius, the molecule of ordinary oxygen is diatomic, containing one electro-positive and one electro-negative atom. On the other hand, active oxygen consists of unpaired atoms which may exist either free or loosely bound together (*lose gebunden*). If electro-negative, these atoms form ozone; if electro-positive, antozone.

Long afterwards it was pointed out by Schuster ([392]) in connection with his investigations on the electrification of gases that the passage of electricity from one molecule to another in a gas is always accompanied by an interchange of atoms composing the molecule. According to this author, physicists of the Faraday-Maxwell school had long considered it probable that the conduction of electricity through gases is due to something similar to the electrolytic conduction in solutions, or, in other words, to the migration of ions under the influence of the current.

Similarly, according to Von Helmholtz and Richarz ([208]) the interesting phenomena observed during the electrification of a steam jet and the influence of certain chemical reactions on condensation occurring in the jet, can only be explained on the supposition that molecular oxygen or the gaseous substances in the immediate vicinity of the jet are dissociated to a greater or less extent into free atoms or ions, or into molecular groups containing free valences, and that these are responsible for the phenomena observed. It has been observed by these and other investigators that when a jet of steam emerges from a small orifice into dust-free air, there is very little if any condensation to be observed within half an inch or so of the orifice. On the other hand, if an electrode from which electricity is escaping be placed near the origin of the jet, dense condensation occurs right up to the orifice, and the jet itself appears colored, the color being due to the scattering of light by a great number of very small particles of water, the diameter of which is very small compared with the wave length of light.

According to J. J. Thomson ([422]), the electricity which escapes into the gas is carried by the charged atoms of the gas, and since in the region immediately around these atoms there will be a very intense electric field there will be a tendency for the steam to deposit in these regions. Thus around these charged atoms there will be very small drops of water, which will scatter the blue light more than the red, so that the jet when seen by transmitted light will appear reddish.

Von Helmholtz and Richarz ([208]) found further that condensation in the steam jet is also brought about by chemical action going on in its neighborhood. The reactions investigated were of the most varied character, involving such processes as the combination of nitric oxide and oxygen, ammonia, and hydrochloric acid, the oxidation of phosphorus, sulphur, and other readily combustible substances. All of these were found not only to effect the condensation of live steam, but also to cause the condensation of other vapors, such as those of alcohol and acetic acid. As a matter of fact so universal is this influence that Von Helmholtz and Richarz proposed to employ the steam jet as a means of detecting ionic dissociation in gases. Thus Von Helmholtz ([207]) found that condensation is effected in the steam jet by various glowing metals, such as platinum foil which had been heated to redness and which had not been artificially electrified. Similarly other metals such as silver, iron, copper, etc., after heating in the flame, color the steam jet for a long time. This is also shown in a high degree by all glowing organic bodies, such as wood, paper, tobacco, and especially by glowing sulphur. It was also proved that the production of smoke has nothing to do with the phenomenon observed. Tobacco smoke, especially, was observed to be without influence. Von Helmholtz and Richarz ([208]) found that condensation in the steam jet could be brought about by a piece of glowing phosphorus at a distance of several decimeters away, and that at this distance it acted remarkably powerfully, but less actively, on the vapors of alcohol, anilin, formic, and acetic acids. They also proved that the thick white clouds which are produced when moist phosphorus is exposed to the air or oxygen do not exhibit this phenomenon when the glowing phosphorus is quenched. So, also, the oxidation products of phosphorus already prepared had no influence on the condensation.

On the other hand, the active agent produced during the slow oxidation of phosphorus diffuses with considerable velocity, even against a current of air. Potassium and sodium burning in contact with water also exert an influence on the steam jet through great distances. As has been observed in the case of phosphorus, the resulting cloud, which consists of finely divided potassium or sodium oxide or hydroxide, is of itself inactive. It is also known that potassium and sodium glow on their freshly cut surfaces as the result of autoxidation. Hence all these phenomena are analogous to those observed in the burning of phosphorus.

According to these observers, it can readily be understood that in all oxidations occurring in atmospheric air there are produced so many substances that it is often difficult or impossible to obtain experimental proof by the exclusion of all other causes, that the action on the steam jet is only brought about by ions, especially those of active oxygen. Of the three substances, however, which are always produced during combustions in air, namely ozone, nitrous acid, and hydrogen peroxide, none were found to have any action on the steam jet.

In this connection it is of interest to note that Meissner ([297]) as early as 1863 had observed that under the excitation of oxygen by electrical induction there is produced, besides ozone, still another substance, which exerts a highly remarkable action on water vapor. Further investigations led him to believe that this substance was Schoenbein's antozone.

According to Von Helmholtz and Richarz ([208]), in all such oxidation processes as effect a condensation of aqueous vapor in the steam jet, the oxygen is dissociated into single atoms (ions), 0^{+}_{+} and 0^{-}_{-}, or into groups of atoms such as $-O-O-$. In terms of the electro-chemical theory both of these kinds of particles have free valences and are carriers of an excess of positive or negative electricity, and hence serve to conduct the current through the gas during electrification, or, as explained by Thomson (see p. 16), they cause a condensation of aqueous vapor when they come in contact with the steam jet. According to Von Helmholtz and Richarz ([208]), chemists have ascribed to active oxygen only a momentary existence; on the other hand, Clausius ([121]), on purely physical grounds, postulated the existence of dissociated atoms of oxygen at least as a transitional form enduring certainly for a short time, and if we judge from their conduct toward steam these particles of active oxygen exist for a sensible interval. By means of tetramethyl-p-phenylene-diamin, which is colored blue by contact with monatomic oxygen (antozone), Wurster ([464]) was able to obtain evidence of the existence of the dissociation products of oxygen in the vicinity of gas flames. Thus Wurster's reagent is instantly colored intensely blue in the Bunsen burner flame; more slowly, but still visibly, in the luminous zone of flames and combustible gases, and also in the flame of burning alcohol. Similarly it was observed by Von Helmholtz (207)[a] that Wurster's paper is colored blue in the neighborhood of a glowing platinum spiral, pointing to the presence of active oxygen in the surrounding atmosphere.

It has also been shown by Elster and Geitel (158) that the electrification of gases is brought about by glowing bodies. They (159, 160, 161, 162) likewise observed that air in which moist phosphorus is oxidizing conducts the electric current, while ozone does not conduct it. Hence they conclude that this conduction is accomplished by ions, or split oxygen molecules, and that the production of ozone must result from the previous splitting of the oxygen molecule. That ozone differs essentially in its conduct from atomic oxygen is also indicated by the fact that ozone itself has no effect on the steam jet, whereas decomposing ozone, like decomposing oxygen, affects it most actively. (*See also* Von Helmholtz and Richarz (208), pp. 194–195, and also Meissner (296).

It is evident, therefore, from these observations and others of similar import, that the dissociation of oxygen, whereby active or ionic oxygen is produced under a great variety of conditions, had been recognized for some time before it was employed by Van't Hoff in explanation of oxygen activation. In reality the idea that in oxygen gas we have an equilibrium represented by the equation, $O_2 \rightleftarrows 2O$, dates from the time of Clausius. On the other hand, we are indebted to Van't Hoff and his coworkers (214, 236), especially to Ewan (169), for experimental proof that in the oxidation of acetic aldehyde the velocity of the reaction is as indicated by the above equation, proportional to the square root of the oxygen pressure, and to Van't Hoff himself for experimental proof than an atom of oxygen is rendered active for every two atoms of phosphorus oxidized, irrespective of the nature of the acid of phosphorus produced.

The fact that oxygen can distribute itself equally between two oxidizable substances, one of which is autoxidizable, while the other is not, is supported by a vast amount of experimental evidence, all of which, to a degree at least, supports Van't Hoff's hypothesis. Thus, to take an actual case studied by Jorissen (234, 235, 236), 131.5 milligrams of tri-ethyl phosphine were found to require 17.8 milligrams of oxygen to completely oxidize it in the sense of this equation,

$$(C_2H_5)_3P + O = (C_2H_5)_3PO.$$

whereas when this same amount of this compound was oxidized in the presence of 2 grams of sodium indigo-sulfonate, 35.3 milligrams of oxygen were consumed, or approximately twice as much. Similar results were obtained by this observer with propionic and benzaldehydes. (*See also* Van't Hoff (215).

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[a] See also Gorup-Besanez (206).

So also Meyer and Recklinghausen (301) and Hirtz and Meyer (212), in their study of the oxidation of hydrogen and carbon monoxide by means of potassium permanganate, have made the interesting observation that approximately as much oxygen is liberated in gaseous form as is absorbed by the reducing substance during the oxidation. These authors are therefore of the opinion that these phenomena probably stand in close relation to the phenomena described by Van't Hoff and his coworkers. The fact that ordinary oxygen dissociates at least to a slight extent into positive and negative ions, even at ordinary temperatures, enables us, according to Van't Hoff, to understand why the oxidation of one substance promotes the oxidation of another. All ions of one kind enter into combination with the autoxidizable substance during the process of autoxidation, thereby leaving the ions of the opposite kind free to combine with a second substance, the acceptor, which ordinarily in the absence of a carrier, will not combine with oxygen at all. Thus in the oxidation of indigo by oxidizing phosphorus, we would have, according to Van't Hoff:

$$O_2 = \oplus + \ominus,$$

and

$$P + \oplus = PO,$$

and

$$\text{Indigo} + \ominus = \text{Isatin};$$

or in general,

$$O_2 = \oplus + \ominus,$$

and

$$A + \oplus = AO,$$

and

$$B + \ominus = BO.$$

According to this view neither A nor B can combine with molecular oxygen, but since this dissociates to a slight extent, even under ordinary conditions, and since $\oplus$ can combine with A, it leaves the other ion, $\ominus$, free to combine with B.

The assumption, however, that the oxygen molecule dissociates into two oppositely polarized atoms is by no means rendered necessary by these facts alone, since the equal distribution of oxygen between two oxidizable substances, one of which functions as the autoxidator and the second only in the capacity of acceptor, can, as we shall see, be equally well if not better explained on the assumption that the autoxidizable substance combines with a molecule of oxygen to form an unstable peroxide, which in turn gives up half of its oxygen to the acceptor, thus:

$$A + O_2 = AO_2$$

and

$$AO_2 + B = AO + BO,$$

and certainly for most, if not all, of the actual cases thus far investigated, such an assumption is, as we shall see, more in harmony with the facts, since the formation of such peroxides as AO_2 rests on indisputable experimental evidence.

From his studies on the electrification of gases, Schuster (392) assumes that under the influence of the electric current the molecules of gaseous substances are broken up at the negative pole. A number of chemists in attempting to account for the activation of oxygen have thought it necessary to assume the decomposition or disruption of the oxygen molecule through the action of oxygen carriers. Thus in 1870, Loew (276) advanced the view that in slow and rapid oxidation the splitting of the oxygen molecule into its atoms occurs. Fudakowsky (177) concurs in this view.

From his study of fermentation, more especially of those changes occurring in putrefaction and anaerobic fermentation, Hoppe-Seyler (217) in 1878, arrived at the notion that oxygen is rendered active through the agency of active (nascent) hydrogen, which in all processes of this kind appropriates to itself an atom of oxygen to form water, thereby leaving the other atoms of oxygen free or in the active condition. Thus,

$$H + H + O_2 = H_2O + O$$

and

$$R + O = RO,$$

where R represents a substance which can not ordinarily combine with molecular oxygen. In many processes of putrefaction and anaerobic fermentation, he observed that hydrogen is a constant product. Furthermore, he found it to be *active*, in the sense that it has the power of accomplishing the reduction of many substances not ordinarily reducible by molecular hydrogen. In other words, Hoppe-Seyler (217) assumes that the nascent hydrogen splits the oxygen molecule, forming water and liberating atomic oxygen, which can then combine with easily oxidizable substances, or if no such substances be at hand, with water to form hydrogen peroxide, or with oxygen to form ozone, or with carbon monoxide to form carbon dioxide, as was shown by Baumann (39).

In the field of pure chemistry many facts of similar import were already known, so that in seeking for an explanation of his own results, Hoppe-Seyler found many analogies to draw upon. For example, as early as 1853, Osann ([illegible]) had made the observation that carbon and platinum which had been charged with hydrogen at the cathode in the electrolysis of dilute sulfuric acid, could effect the reduction of silver compounds in solution, etc., and somewhat later Beketoff (42) had shown that copper could be reduced from solutions of copper sulfate and silver from solutions of silver nitrate by passing hydrogen through such solutions containing a piece of platinum foil. On the other hand, such solutions, in the absence of platinum, suffered no reductions in the current of hydrogen alone. Then followed the beautiful discovery of Graham ([197–198]) in 1866–68, that platinum and particularly palladium, and other metals to a slight extent, have the power of absorbing considerable amounts of hydrogen under the influence of moderate amounts of heat, or as the result of electrolysis. Furthermore, the hydrogen in these metallic combinations was recognized as active, in the sense that it could reduce iodine to hydriodic acid, ferric salts to ferrous salts, etc., changes which can not be effected by ordinary molecular hydrogen in the cold. According to Graham, the hydrogen under these conditions becomes polarized, its affinity for oxygen thereby being greatly increased. Such hydrogen, for example, was found to combine with oxygen even in the cold, with the production of water. Now in the course of his own investigations Hoppe-Seyler found that freshly prepared palladium hydride has the power of reducing copper sulfate to metallic copper, that it decolorizes a solution of indigo, and reduces potassium permanganate in neutral solution to manganese dioxide, or in acid solution to a manganous salt; that it converts quinone into quinhydrone and changes oxyhemoglobin to methemoglobin. He found further that these powerful reductions are brought about only when the palladium hydrogen compound is fresh; if allowed to stand it loses these properties, while it is still found to contain hydrogen.

These reducing actions correspond entirely to those exhibited on dissolving zinc or tin in dilute acids; in other words, they are effected by nascent or active hydrogen. According to Hoppe-Seyler the most interesting reduction which can be effected by active hydrogen is that of molecular oxygen to water, whereby $-OH$ or $-O-O-H$, and finally water and active oxygen, are formed successively. He found further that if one shakes some palladium foil saturated with hydrogen with a dilute starch paste containing potassium iodide and air, there is produced in a few moments a dark-blue solution of the iodide of starch. In other words, through the influence of the hydride of palladium, potassium iodide is oxidized by the oxygen of the air, a change which ordinarily takes place only with extreme slowness. If now the foil be boiled with water and heated to redness to expel the hydrogen, it no longer has the power to form the iodide of starch when shaken with a solution of potassium iodide and starch in contact with air.

Similarly, indigo is oxidized to isatin and ammonia to ammonium nitrite, benzene to phenol and toluene to benzoic acid, when these several substances in aqueous solution are shaken with air and palladium hydride. All of these changes go to show that we have here to deal with very vigorous oxidations, and it can not be doubted that they are brought about indirectly by active hydrogen. According to Hoppe-Seyler there is no probability of another explanation than that the active hydrogen renders the oxygen active, and since it, the hydrogen, combines with oxygen to form water, the process can scarcely be conceived otherwise than that when active hydrogen appropriates unto itself one atom of oxygen of the oxygen molecule to form water, it sets free the other atom of oxygen, thereby rendering it active, and that just as the free atom of hydrogen can not remain free, so this atom of oxygen can not remain free, and hence when no other oxidizable substance is present, it unites with the water to form hydrogen peroxide, or with molecular oxygen, O_2, to form ozone. The action of hydrogen upon indifferent oxygen corresponds to the action thereupon of many other substances having a strong affinity for oxygen, such, for example, as magnesium, phosphorus, etc. Hence it is that when magnesium burns in the air nitrous acid is produced (Kämmerer[237]), and in the slow oxidation of phosphorus one atom of oxygen is rendered active for every two atoms of phosphorus oxidized. This latter change Hoppe-Seyler represented in the following manner:

$$2P + 3H_2O + 2O_2 = 2(PO_3H_3) + O,$$

so that for every two atoms of phosphorus oxidized at least one atom of oxygen is rendered active. According to Hoppe-Seyler, the observation by Schoenbein that hydrogen peroxide is formed by shaking zinc dust or iron powder with air and water can scarcely be interpreted otherwise than as resulting from the reduction of indifferent oxygen by the metal. Thus in the oxidation of iron in the presence of water we would have, according to Hoppe-Seyler, the following reaction:

$$Fe + O]:[O + H_2O = FeO + H_2O_2.$$

According to this author, however, none of these processes exhibit these changes as clearly and as simply as palladium hydride. Hoppe-Seyler saw, therefore, in this remarkable compound the chemical prototype of those complex unstable hydrogen compounds resulting from the anaerobic changes occurring in living matter, the decomposition of which gives rise to active hydrogen, and upon the conduct of palladium hydride this author based his conclusions respecting animal oxidation. During putrefaction, hydrogen is produced, and while in the absence of air powerful reductions occur, in the presence of air, powerful oxidations also occur in the putrefying liquid, particularly in the upper or exposed portions thereof. He was also of the opinion that similar changes occur in all living cells. The weak point in this theory of oxygen-activation is that it accounted for the formation of hydrogen peroxide in certain oxidations on the supposition that this compound is formed by the oxidation of water by an atom of active oxygen. Thus he explained the formation of hydrogen peroxide during the oxidation of metals in the following manner:

$$M + O_2 = MO + O,$$

and

$$H_2O + O = H_2O_2.$$

The principal objections which have been urged against this theory are, first, that it fails to take into account the formation of peroxides other than hydrogen peroxide as the result of processes of autoxidation, and, second, that it accounts for the formation of hydrogen peroxide in processes of autoxidation on the assumption that this compound results from the oxidation of water. It is now known that many peroxides other than hydrogen peroxide are produced during processes of autoxidation, and it was pointed out by Weltzien (446, 447) as early as 1860 that hydrogen peroxide can not be regarded as oxidized water. (*See also* Bach (18.) While it was afterwards claimed by Richardson (343, 347, 348) that water is oxidized during the oxidation of ether by oxygen in sunlight, apparently the precise conditions for accomplishing this oxidation have never been described, and Dunstan and Dymond (153) have shown that hydrogen peroxide is never produced by the action of oxygen on water under the influence of light and heat, even in the presence of dilute sulfuric acid. We know now that hydrogen peroxide is formed during the autoxidation of various substances, sometimes as the primary product of the oxidation of labile hydrogen atoms or hydrogen ions; more frequently as a secondary product resulting from the hydrolysis of another peroxide previously formed during the autoxidation, but never by the oxidation of water.

In a series of remarkably interesting and suggestive communications extending over a period of eleven years, from 1882 to 1893, and published for the most part in the Berichte der Deutschen Chemischen Gesellschaft, Mauritz Traube (431) laid the foundations of the peroxide theory of oxidation and enriched the nomenclature of the subject with many valuable terms, such as "autoxidation," "autoxidizable," "holoxide," etc. The clue to an understanding of Traube's peroxide theory of oxidation is to be found in the fact that water is necessary for most if not all oxidations proceeding spontaneously at ordinary temperatures and that hydrogen peroxide is produced as one of the products in all or at least in nearly all of those oxidations that are effected by molecular oxygen. He (433) made the interesting observation that pure metallic sodium retains its bright luster for forty hours in an atmosphere of dry oxygen, whereas it is instantly tarnished the moment that a trace of moisture is admitted.

He also discovered that in perfectly pure air-free water, metals like zinc and iron retain their luster undiminished, whereas if air be admitted they soon rust. Evidently, therefore, two factors are concerned in the rusting of metals, viz. water and oxygen.

It was also recognized about this time that water is essential to the oxidation of combustible gases. Dixon (110) had observed that a mixture of perfectly dry carbon monoxide and oxygen does not explode under the influence of the electric spark, nor do they combine when passed over red-hot platinum gauze, and Traube (134) himself made the observation that a flame of burning carbon monoxide is extinguished on being brought into perfectly dry air. He therefore reached the conclusion that "no substance can act upon dry oxygen at ordinary temperature." (Traube (133), p. 1881.) Other chemists, among them Nef (311) and Armstrong (13), have arrived at essentially similar conclusions respecting the necessity for water in many such processes of oxidation, and recently Dunstan, Jowett, and Goulding (154) have found that both water and oxygen, the former in the liquid condition, are necessary for the rusting of iron. Cushman (128) also, in a recent communication on the "Corrosion of Iron," states that while not agreed as to the mechanism of the process, all investigators are agreed that both oxygen and water are essential to the rusting of iron.

That hydrogen peroxide is formed during the oxidation of oxidizable substances by molecular oxygen was known even to Schoenbein (*see* Traube (131), supra), and since his time instances of its formation during oxidation had greatly accumulated in chemical literature. When, for example, finely divided zinc is shaken with air and water, the metal is gradually oxidized and hydrogen peroxide is found in considerable quantities in the solution. These observations on the production of hydrogen peroxide as the result of oxidation by molecular oxygen acting in the presence of water were greatly extended by Traube. Thus he (132) observed its production in large amounts at the cathode during electrolysis when oxygen or air is passed through the solution surrounding the negative pole, whereas it is never produced by the action of molecular hydrogen on the oxygen liberated at the anode. Schuller (391) had shown that hydrogen peroxide results from the burning of hydrogen, and on repeating these experiments Traube (135) succeeded in obtaining as much as 0.0108 gram of hydrogen peroxide by the burning of 1 liter of hydrogen. Traube (134) also made the interesting observation that hydrogen peroxide is produced by allowing a flame of burning carbon monoxide to impinge on the surface of water. These and many facts of similar import led him to believe that in all oxidations it is the molecule of oxygen and not the atom which first enters into combination with the autoxidizable substance.

To Traube, therefore, we owe the introduction into the science of a number of distinctly new ideas respecting oxidation phenomena. These are as follows:

First. That in all processes of autoxidation the oxygen molecule as a whole combines with the oxidizable substance, or with the hydrogen of water under the influence of the oxidizable substance, to form a peroxide (holoxide). Thus when hydrogen burns in air or oxygen, hydrogen peroxide is the primary product of the oxidation, the formation of water resulting from the reduction of the peroxide by the action of the hydrogen; thus:

$$H_2 + O_2 = H_2O_2,$$

and

$$H_2O_2 + H_2 = 2H_2O.$$

Second. That water actively participates in all or at least in the greater number of autoxidations, and that hydrogen peroxide is formed as a primary product of such autoxidations. When, for example, zinc oxidizes at ordinary temperatures in the presence of water the following changes occur:

$$\mathrm{Zn} + \left.\begin{matrix}\mathrm{HO}\\ \mathrm{HO}\end{matrix}\right]\!\begin{matrix}.\\ .\end{matrix}\!\left[\begin{matrix}\mathrm{H}\\ \mathrm{H}\end{matrix}\right. + O_2 = \mathrm{Zn(OH)_2} + H_2O_2.$$

Similarly, according to Traube, traces of moisture are necessary for the burning of carbon monoxide for the following reasons:

$$\mathrm{CO} + \left.\begin{matrix}\mathrm{HO}\\ \mathrm{HO}\end{matrix}\right]\!\begin{matrix}.\\ .\end{matrix}\!\left[\begin{matrix}\mathrm{H}\\ \mathrm{H}\end{matrix}\right. + O_2 = H_2CO_3 + H_2O_2,$$

and

$$CO + H_2O_2 = H_2CO_3,$$

and

$$2H_2CO_3 = 2CO_2 + 2H_2O.$$

Third. That the phenomenon of oxygen-carrying is due primarily to the oxidation of the second oxidizable substance, the acceptor, by the hydrogen peroxide resulting from the oxidation of the autoxidizable substance (the carrier) in the presence of water. When, for example, palladium hydride is shaken with air and water large amounts of hydrogen peroxide are formed. If, however, a second oxidizable substance is present, such as carbon monoxide or potassium iodide, it, as well as the hydrogen of the palladium hydride, is oxidized for the reason that under the influence of the palladium these are oxidized by hydrogen peroxide; thus:

$$\begin{matrix}\mathrm{Pd_2H}\\ \mathrm{Pd_2H}\end{matrix} + \begin{matrix}\mathrm{HO.H}\\ \mathrm{HO.H}\end{matrix} + O_2 = 4\mathrm{Pd} + 2H_2O + H_2O_2,$$

and

$$CO + H_2O_2 = H_2CO_3,$$

or

$$2KI + H_2O_2 = 2KOH + I_2.$$

Fourth. Contrary to the views of Hoppe-Seyler it is not the oxygen molecule which is decomposed in autoxidations, but the molecule of water, whereby atomic hydrogen is formed, which then combines directly with the molecular oxygen to form hydrogen peroxide. Digramatically the essential points of difference between these two theories of oxidation may be represented as follows:

$$M+\mathrm{O}]\!:\![\mathrm{O}+\mathrm{H_2O}=M\mathrm{O}+\mathrm{H_2O_2}\ \text{(Hoppe-Seyler)},$$

and

$$M+\left.\begin{matrix}\mathrm{HO}\\ \mathrm{HO}\end{matrix}\right]\!:\!\left[\begin{matrix}\mathrm{H}\\ \mathrm{H}\end{matrix}\right.+\mathrm{O_2}=M(\mathrm{OH})_2+\mathrm{H_2O_2}\ \text{(Traube)}.$$

The weak point in Hoppe-Seyler's theory is that it explains the formation of hydrogen peroxide by the oxidation of water. On the contrary, everything points the other way, viz, that water results from hydrogen peroxide by loss of oxygen, either as the result of decomposition or through the action of oxidizable substances.

The objection to Traube's theory is that it has not been possible to prove the presence of hydrogen peroxide among the products of all autoxidations. Thus Cushman (128) obtained no evidence of it in his recent study of the corrosion of iron. Traube, however, explains its absence in certain autoxidations on the ground that it is decomposed as fast as formed by the other substances produced during the oxidation. Thus in the case of the slow oxidation of iron it is probable that the hydrogen peroxide resulting from the first phase of the oxidation is partly consumed in the further oxidation of the ferrous hydroxide to iron rust. It should also be borne in mind that hydrogen peroxide is also decomposed into water and oxygen by most, if not all, of those substances whose oxidation it can accomplish, so that, all things considered, it is not surprising that it should occasionally be found to be absent from solutions in which autoxidation processes are taking place.

During recent years the peroxide theory of oxidation has been considerably extended through the labors of Bach, Engler, and Manchot, and their coworkers in this field. In the main the work of these several observers has consisted in the extension and elaboration of Traube's peroxide theory of oxidation. Thus in 1897 Bach (18) investigated the slow oxidation of a large number of substances and arrived at the conclusion that readily oxidizable substances combine with partially dissociated molecular oxygen, $-\mathrm{O}-\mathrm{O}-$,[a] to form peroxides, and that these latter substances when once formed promote and accelerate the oxidation of any other less readily oxidizable substances that may happen to be present. He also came to the conclusion that the oxidizing ferments of the blood are in reality readily oxidizable substances, having a special aptitude for forming peroxides. Similar conclusions have been reached by other chemists respecting the nature of the plant oxidases.

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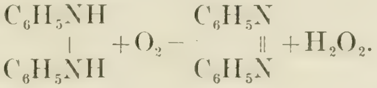
[a] *See* von Helmholtz and Richarz (208).

According to Ostwald (317) Bach's theory of slow oxidation derives support from the energy relations existing between the combining substances and the intermediate and final products of the combustion. He (Ostwald) is also of the opinion that the production of unstable intermediate products is not be looked upon as an uncommon occurrence, but rather as the rule, and that as a general thing in chemical processes it is not the most stable products which are first produced, but ofttimes the most unstable.

More recently Manchot and Herzog (282, 283) have quantitatively studied the oxidation of indigo-white, hydrazobenzene, and a number of other complex organic compounds in alkaline solution, by air or oxygen. All of their results have gone to show that as much oxygen may be obtained from the metallic peroxides resulting from the oxidation as enters into the oxidation of the oxidizable compound itself. In other words, they found that one-half of the total oxygen consumed in such processes went to oxidize the autoxidizable substance, while the other half went to form hydrogen peroxide. Thus in the case of hydrazobenzene we would have



$$\begin{array}{c} C_6H_5NH \\ | \\ C_6H_5NH \end{array} + O_2 = \begin{array}{c} C_6H_5N \\ \| \\ C_6H_5N \end{array} + H_2O_2.$$

As the oxygen molecule is composed of two atoms, he also reached the conclusion that a molecule of oxygen either united with the oxidizable substance to form a primary oxide, which is subsequently decomposed by water into a simpler oxide and hydrogen peroxide, or, as in the case of indigo-white, the molecule of oxygen unites with the hydrogen of the leuco-compound, forming indigo and hydrogen peroxide. If the oxidation take place in a solution of barium hydroxide, the hydrogen peroxide is removed as fast as formed in the form of barium peroxide, equivalent amounts of indigo and barium peroxide being formed, thus:

$$InH_2 + O_2 + Ba(OH)_2 = In + BaO_2. + 2H_2O.$$

In case the hydrogen peroxide is not removed in some insoluble combination, it reacts with a second molecule of indigo-white, in which case we would have ultimately two molecules of indigo formed, thus: [a]

$$InH_2 + O_2 = In + H_2O_2,$$

and

$$InH_2 + H_2O_2 = In + 2H_2O.$$

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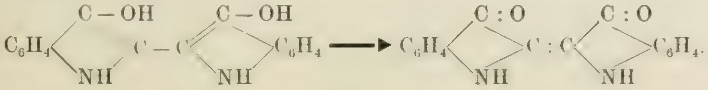
[a] Falk (170) in discussing the oxidation of indigo-white has offered the interesting and plausible suggestion that indigo-white may be looked upon as a phenol, which on oxidation passes to a quinone, thus:



These experiments afford quantitative proof of the peroxide theory of oxidation.

A similar case has been studied by Bamberger (34), who showed that when an aqueous solution of phenylhydroxylamin is exposed to the air it is oxidized to nitrosobenzene with the formation of hydrogen peroxide, thus:

$$C_6H_5NHOH + O_2 = C_6H_5NO + H_2O_2.$$

Thus three grams of phenylhydroxylamin in 40 grams of water yield, after treatment with a current of air for seventy hours, 2.5 grams of azoxybenzene.

Finally, during the last ten or twelve years Engler and his coworkers (164, 165) have done a great deal to extend the peroxide theory of oxidation. According to these authors every oxidation consists primarily in the union of molecular oxygen with the substance undergoing the oxidation, and that, contrary to Traube, the primary product of the autoxidation is not necessarily hydrogen peroxide but a peroxide of the substance undergoing oxidation. Thus when rubidium burns in air it is converted quantitatively into rubidium peroxide—

$$Rb + O_2 = RbO_2,$$

and, as shown by Baeyer and Villiger (33), when benzaldehyde is exposed to oxygen or air it is first converted into benzoyl-hydrogen peroxide, thus:

$$C_6H_5CHO + O_2 = C_6H_5CO.O.OH.$$

Engler and his coworkers have also pointed out that hydrogen peroxide, so frequently encountered in processes of autoxidation, may be produced in several entirely different ways; first, as the primary product of the autoxidation—as, for example, the burning of hydrogen or by the action of oxygen on the hydrogen liberated at the cathode during electrolysis, or in the oxidation of substances like indigo-white and hydrazobenzene, which contain labile hydrogen atoms, thus:

$$\begin{array}{l} H- \\ H- \end{array} + \begin{array}{l} -O \\ -O \end{array} = H_2O_2,$$

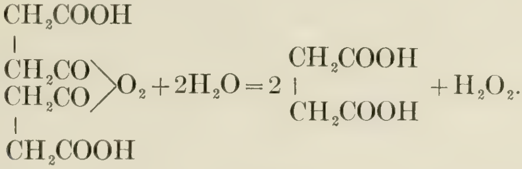
and

$$\mathit{In}H_2 + O_2 = \mathit{In} + H_2O_2;$$

or, as Traube pointed out, it may result from any autoxidation in which water is essential to the oxidation; thus:

$$Zn + \left.\begin{array}{l} HO \\ HO \end{array}\right]\!\cdot\!\cdot\!\left[\begin{array}{l} H \\ H \end{array}\right. + O_2 = Zn(OH)_2 + H_2O_2.$$

According to Engler, the true autoxidator in such processes is the hydrogen ion, while he looks upon the zinc as the pseudo-autoxidator. Secondly, as pointed out by Engler, hydrogen peroxide is frequently a secondary product resulting from the decomposition or hydrolysis of the primary peroxide by the action of water. Thus, Clover and Houghton (122) have observed that succinic peroxide acid is readily hydrolyzed by water, yielding succinic acid and hydrogen peroxide, in the sense of the equation—



$$\begin{matrix} CH_2COOH \\ | \\ CH_2CO \\ CH_2CO \\ | \\ CH_2COOH \end{matrix} \!\!>\! O_2 + 2H_2O = 2 \begin{matrix} CH_2COOH \\ | \\ CH_2COOH \end{matrix} + H_2O_2.$$

Finally, Engler and Weissberg (164), have laid considerable stress upon the idea that dissociation or the liberation of free valences, both in the oxygen molecule and in the substance undergoing autoxidation, are necessary for autoxidation processes, and in this way they explain the effect of heat and light in accelerating oxidations and also account for the ease with which unsaturated organic compounds undergo oxidation. They assume, with von Helmholtz and Richarz (208) and Bach (18), a partial dissociation of the oxygen molecule, resulting in the complex $-O-O-$, thereby enabling them to account for the direct addition of the whole molecule of oxygen to the oxidizing substance.

In the light of these considerations the phenomenon of oxygen-activation is easily explained. According to Engler and his followers it is due primarily to the oxidation of the second substance—the acceptor—by the peroxide (moloxide) resulting from the autoxidation of the carrier. Thus, when an autoxidizable substance, A, finds itself in contact with oxygen and a second oxidizable substance, B, the following changes would occur:

$$A + O_2 = AO_2,$$

and

$$AO_2 + B = AO + BO.$$

In this way a substance incapable of combining directly with oxygen may be oxidized through the intervention of another substance, and a given quantity of oxygen equally distributed between them. To take the case of the oxidation of benzaldehyde and the changes which may be accomplished through the oxidation of this compound at ordinary temperatures in the air. When exposed to the air this compound is converted into benzoyl-hydrogen peroxide (*see* Baeyer and Villiger (33))—

$$C_6H_5CHO + O_2 = C_6H_5CO-O-O-H.$$

If allowed to remain in contact with water, benzoyl-hydrogen peroxide is hydrolyzed with the production of benzoic acid and hydrogen peroxide—

$$C_6H_5CO.O.OH + HOH = C_6H_5COOH + H_2O_2.$$

On the other hand, if allowed to remain in contact with a second molecule of benzaldehyde, it oxidizes the second molecule of the aldehyde with the production of two molecules of benzoic acid.

$$C_6H_5CO.O.OH + C_6H_5CHO = 2C_6H_5COOH;$$

or finally, if an oxidizable substance, such as indigo, be present, it oxidizes the indigo with the production of benzoic acid and isatin, thus—

$$C_{16}H_{10}N_2O_2 + C_6H_5CO.O.OH = 2C_8H_5NO_2 + 2C_6H_5COOH.$$

Hence when a solution of indigo is shaken with benzaldehyde both are oxidized, whereas the indigo alone is unchanged by molecular oxygen.

CHAPTER II.

THE OXIDIZING FERMENTS.

In its present shape, therefore, the peroxide theory of oxidation accounts for the phenomena of autoxidation and oxygen carrying upon the supposition that spontaneously oxidizable substances have the power of combining with partially dissociated molecules of oxygen to form peroxides. These peroxides may then react either with additional amounts of the autoxidizable substance itself or with some other substance to form simpler oxides. Thus, it may happen that as the result of its oxidation one substance, *A*, may effect the oxidation of a second substance, *B*, which latter is not directly oxidizable under ordinary conditions. In this way the modern theory of oxidation affords a simple explanation of the formation of peroxides and of the phenomenon of oxygen carrying.

The numerous applications of this theory are by no means confined to the inorganic world. In the life cycle of the plant and animal we meet with many changes which, in so far as oxygen is involved, find their simplest explanation in terms of this hypothesis. The affinity for oxygen which is displayed not only by the warm-blooded animals, but even by plants, is often remarkable. That such is the case may be gathered from the fact that Phipson (320) in 1896 employed one of the higher fungi, *Agaricus atramentarius*, as an agent wherewith to effect the analysis of atmospheric air. It has so happened, therefore, that in the history of our science both a mouse and a mushroom have been employed as oxygen absorbents wherewith to determine the quantity of oxygen in atmospheric air. During the absorption of oxygen by plants various substances are produced which are capable of effecting oxidations which ordinarily can not be brought about by molecular oxygen alone, such, for example, as the bluing of guaiacum, the conversion of hydroquinone into quinone, the liberation of iodine from potassium iodide, etc. Like many of the chemical agents of the living cell, these powerful oxidizing substances are characterized by great instability. They are destroyed by heat and mineral acids and by various poisons, such as hydrogen cyanide, sulphur dioxide, phenylhydrazin, etc. It is claimed by some observers that they act catalytically, and hence are believed to partake of the nature of ferments. These substances are the *oxidases*,[a] or oxidizing ferments, and closely associated with this remarkable group of substances are the *peroxidases* and *catalases*, which have the power of decomposing hydrogen peroxide or rendering active the oxygen which it contains.

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[a] The following general references to the literature of this subject will be found of interest in this connection: Remarques sur les matières oxydantes, que l'on peut rencontrer chez les êtres vivants, by Bourquelot (85); Ueber Oxydationsfermente, by

While in many instances, as pointed out by Wender (448), the precise rôle of these catalysts in the life of the cell has not as yet been determined, it is known that they are very widely distributed in the plant and animal kingdoms and that they are concerned in many important bio-chemical processes. Thus, according to Tolomei (423) and Martinand (286) the oxidation of the coloring matter of the grape and the production of those aromatic substances which confer upon old wine its delightful aroma and taste is due to the action of oxidases which are present in the grape and in the yeast. Aso (15) has shown that oxidases are concerned in the curing of tea. They probably also play a part in the curing of tobacco, and in the change of color of any vegetable tissue from green to brown, which results finally in the production of humus substances. According to Grüss (201), the oxidases play an important rôle in the formation of starch grains. Hahn (204) found an oxidase in *Arum maculatum* which had the power of oxidizing grape sugar and which apparently assists in intramolecular respiration, and Raciborski (337) is of the opinion that the vegetable oxidases play the same rôle in the vasculiferous plants as that played by hemoglobin in the higher animals and hemocyanin in the lower animals, viz, that of the oxygen-laden vehicle of respiration. Woods (462) has shown that oxidases are responsible for the etiolation of green leaves.

Palladin (317a) and also Miss Wheldale (449a) are of the opinion that anthocyanin, the red pigment of certain flowers, results from the action of an oxidase on some colorless, chromogenic substance contained in the flower.

The oxidases are by no means confined to the plant, but are found in many animal tissues, where they are assumed to play an equally important rôle in oxygen metabolism. Thus, Dubois (147) attributes the phosphorescence of phosphorescent animals and plants to the action of an oxidase to which he has given the poetic name of "Luciferase," and this same observer (148) has traced the formation of a purple dye by the mollusc *Murex brandaris*, to the action of an oxidase which he calls "Purpurase." Carnot (111) found that the saliva and other secretions contain certain oxidases. Portier (330) has pointed out that oxidases are concentrated in the epidermal and exposed structures of

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Steudel (411); Zur chemischen Erkenntnis biologischer Oxydationsreaktionen, by Engler and Herzog (165); Contribution a l'Etude des Applications Therapeutiques des Oxydases et des Metaux Ferments, by Pierre Sée ([illegible]); Die Bedeutung der Katalyse für die Medicin, by H. Schade ([illegible]); Les Oxidations de l'Organisme, by Enriquez and Sicard ([illegible]); Ueber die Oxydationsfermente der Leber, by Jacoby ([illegible]); Die Oxydasen, by Neumann Wender (448); The Properties and Classification of the Oxidizing Enzymes, and Analogies between Enzymic Activity and the Effects of Immune Bodies and Complements, by Moore and Whitley ([illegible]); Les Ferments Oxydants, by Chodat (116).

the organism. He is of the opinion, therefore, that their physiological rôle both in animals and plants is one of defense against invasion by micro-organisms. In this connection Sieber (402) has shown that the oxidases have the power of destroying toxins. According to her observations, the fibrin of the blood of a normal horse gives no oxidase reactions, whereas the fibrin obtained from the blood of a horse immunized against diphtheria gives a blue coloration with guaiacum, indicating the formation of oxidases in the blood as the result of immunization. As we shall see, one of the oxidases, tyrosinase, is the active agent in the production of melanins, and hence of profound significance in its relation to certain pathological conditions of melanogenesis in man. In this connection Hougonenq and Paviot (220) claim to have found that certain malignant tumors give the guaiacum reaction. Still other facts of interest, pertaining to the occurrence of the oxidases or to their mode of action, are set forth in the following pages.

THE GUAIACUM REACTION.

Our first knowledge of the oxidizing ferments is closely associated with what is known as the guaiacum reaction, viz, the production of a blue coloring matter when gum guaiacum or its tincture is treated with certain oxidizing agents. According to Binz (68) our knowledge of guaiacum dates back to the sixteenth century when in 1508 it was first imported into Spain from Santo Domingo as a remedy for syphilis by Consolvus Ferrand, himself a syphilitic, soon after which it became known all over Europe as an antisyphilitic. (*See* Monograph of Ulrich von Hutten, De Guajaci Medicina et Morto Gallico; Liebermann, Mainz, 1519, p. 74).

That guaiacum resin becomes bluish-green in color on standing in the air and light and the liquid around the stoppers of bottles containing tincture of guaiacum generally acquires a bluish-green color are matters of everyday experience to those who have ever had occasion to use these substances. In 1804 Wollaston (461) was the first to show that this color change on the part of guaiacum is brought about by the air under the influence of light. According to this author, rays of light which cause the emission of oxygen by silver chloride cause its absorption by gum guaiacum. A few years later William Brande (94) showed that guaiacum becomes blue in pure oxygen gas more rapidly than in air.

Shortly afterwards a number of interesting observations on the bluing of guaiacum were made by the French pharmacists. Thus it was pointed out by Boullay (73) that a mixture of simple sirup, distilled water, gum arabic, and tincture of guaiacum takes on the color of a suspension of verdigris, and further that certain dentifrices containing guaiacum, when taken into the mouth, become intensely blue or green. This change of color had been attributed to impurities in the mouth cavity; he proved, however, that gelatin and albumin produced similar changes of color with alcoholic guaiacum. He therefore reached the conclusion that this change of color is without doubt due to the albumin of the saliva, since no change of color is observed if the mouth be deprived of saliva by previous washing with water. Similarly, according to Mare (285), it was pointed out by Goettling that a mixture of guaiacum resin, gum arabic, sugar, and water of peppermint becomes sensibly blue; that certain acids impart a bluish tint to guaiacum resin, and that this same change is brought about by sweet spirits of niter.

In 1810 Planche (326) in a note on the sophistication of Jalap Resin and a means of recognizing the same, calls attention to the fact that guaiacum resin takes on an intense blue color when exposed for a few minutes to the vapors of nitric acid, and further that the fresh root of the horse-radish also has the power of turning the tincture of guaiacum resin blue. In order to show this, he says, it is only necessary to plunge a little piece of the fresh root into a glass containing the tincture of guaiacum, when little by little the liquid acquires the color of indigo in sulfuric acid. In 1819 Taddey (sometimes spelled Taddei) (416), having had occasion to knead together several species of gum resins and resins proper with different sorts of flour, observed that the mixture of wheat flour and guaiacum becomes blue, especially after water has been added to the mixture exposed to the air. At his suggestion Rudolphi (416) followed up the investigation of this subject. He found that a mixture of guaiacum resin with pure starch does not develop a blue color when moistened and exposed to the air, nor is this blue color developed by other vegetable materials which do not contain *zimôme*, the name proposed by Taddei for the constituent of gluten insoluble in alcohol. He also observed that guaiacum is not colored, or at least only slowly, by flours poor in gluten and that it is not colored by those flours in which the gluten has suffered any great alteration. He observed further that when gluten or pure *zimôme* is mixed with guaiacum it develops a superb blue color instantly, but that such a mixture only develops this color in atmospheric air. Rudolphi therefore proposed to make use of guaiacum as a reagent for judging of the purity and quality of different kinds of wheat flour, and conversely, he recommended wheat flour as a reagent for testing the purity of guaiacum resin.

In 1820 Planche (327) undertook to determine the nature of the substance which produces the blue coloration with guaiacum. He conceived the idea that air and light had nothing to do with the bluing of guaiacum for the reason that if this change of color is due solely to the action of air and light, why is it, he asks, that when guaiacum resin is mixed with certain substances it is colored blue or green, whereas when mixed with other substances its natural color is not altered? In attempting to answer this question he was led to test the conduct of a large number of substances toward guaiacum, among them a large number of roots, fresh and dried, several gums, milk, soap, etc. Among the fresh roots tested the following were found by this observer to blue guaiacum: Comfrey (*Symphytum consolida*, L.), dandelion (*Leontodon taraxacum*), common iris (*Iris germanica*), chicory (*Cichorium intybus*), thistle (*Eryngium campestre*), white water lily (*Nymphyaea alba*), potato (*Solanum tuberosum*), bryony (*Bryonia dioica*), elecampane (*Inula helenium*), marshmallow (*Althea officinalis*), carrot (*Daucus carota*), licorice (*Glycyrrhiza glabra*), turnip (*Napis sativa*), burdock (*Arctium lappa*), colchicum (*Colchicum autumnale*), soapwort (*Saponaria officinalis*), scurvy grass (*Cochlearia officinalis*), fumitory (*Fumaria officinalis*), figwort (*Scrophularia officinalis*), sorrel (*Rumex acetosa*), viper's grass (*Scorzonera hispanica*), asparagus (*Asparagus officinalis*), borage (*Borago officinalis*), angelica (*Angelica archangelica*), onion (*Allium caepa*), wild radish (*Cochlearia armoracia*, L.), little radish (*Raphanus sativus*). The fresh root of the chicory, especially in the fall of the year, was found to give with guaiacum a magnificent blue color. So also the fresh root of the water lily produced a very intense coloration at first, which faded rapidly on standing. For the most part the dried roots gave no color with guaiacum. Neither did the fresh roots of the following plants: Patience (*Rumex acutus*, L.), male fern (*Polypodium filix mas*), strawberry (*Fragaria vesca*). Furthermore he found that light was without influence on the development of the blue color of guaiacum by roots capable of effecting this change. Thus the parsnip caused the bluing of guaiacum in the dark. Similarly when guaiacum and white soap were mixed together there resulted a greenish mass which finally took on the color of verdigris, and the interior of the mass acquired a deep blue color. Obviously the light could not have been responsible for this increase of the color in the interior portions of the mixture. He also observed that milk has the power of bluing guaiacum, and that this change of color takes place in a vacuum. He concludes, therefore, that in the bluing of guaiacum by milk, air is not responsible for the change of color. On boiling, however, milk lost its power to blue guaiacum, and in general, those substances, gums and plant roots, which have the power of bluing guaiacum, lose this power by heat. Thus the potato, parsnip, and beechnut (*Fagus castanea*), cooked in closed vessels in their own juices, do not react with either the powdered resin or the tincture. He therefore concluded that the substance or substances causing the bluing of guaiacum are volatile and possibly condensable. He was therefore led to test the conduct of the distillate obtained from milk toward guaiacum, with the result, however, that no change of color was produced. He therefore had to renounce the idea that the principle responsible for the bluing of guaiacum is volatile. He was led to believe, therefore, that this particular species of cyanogen (*cyanogene*), which is responsible for the bluing of guaiacum, whatever its nature, is absorbed by certain substances which in its ordinary state permit of this action, but that when exposed to certain temperatures it enters into new combinations and obeys other laws which do not permit of its coloring guaiacum.

To anyone at all familiar with the oxidizing ferments it is evident how close Planche came to the discovery of this remarkable group of substances. As a matter of fact he seems to have been the first to encounter them, and when he proved that heat destroys the power of milk and certain plant juices to blue guaiacum, he discovered one of their most remarkable characteristics, and had the state of knowledge regarding oxidation and fermentation been at this time what it was in the time of Schoenbein and Traube, there is scarcely room for doubt that he, and perhaps Taddei also, would have recognized the true nature of these substances and the part which they play in oxidation processes.

From the time of Planche to that of Schoenbein and Traube there appears to have been but little advance in our knowledge of the guaiacum reaction, except the discovery of various substances, organic and inorganic, which effect this change of color to a greater or less extent. Thus Regimbau, (340, 341) in letters to Planche, described the bluing of pills containing guaiacum resin, mercuric chloride, and white soap, and advanced the view that this bluing was due to the liberation of chlorine from the mercuric chloride, a view which Planche pointed out as erroneous; and Lodibert (274) reported results with dentifrices similar to those observed by Boullay (73). Pelletier and Deville (318) also published a paper on guaiacum in 1844, in which, however, there is nothing of any particular interest.

The further elucidation of the guaiacum reaction and the final discovery of the oxidizing ferments awaited the discovery of ozone and the renewed interest in the general subject of fermentation awakened by the writings of Pasteur and Liebig. In this connection it will be recalled that ozone was first recognized as a definite substance by Schoenbein (368, 369). In 1845 Schoenbein (370) showed that guaiacum is colored blue by ozone. The interest attaching to Schoenbein's earlier observations on the bluing of guaiacum by ozone is such in this connection that I shall present the subject in practically the author's own words. In his "Note on guaiacum resin" he goes on to say that—

> It has long been known that guaiacum resin under certain conditions becomes blue and that chlorine has the power of producing this change of color. I have found that bromine and iodine produce the same change. In consequence of the close similarity which chlorine, bromine, and iodine exhibit to ozone, one would naturally

expect that the latter substance would blue guaiacum resin, and my experiments have shown that such is really the case. If one brings a strip of filter paper which has been saturated with guaiacum tincture into strongly ozonized air, prepared by means of phosphorus, the paper at once takes on a blue color. As a matter of fact, guaiacum resin is as sensitive a reagent for ozone as the potassium iodide and starch mixture. If one allows the strips to remain for a somewhat longer time in the ozonized atmosphere, the color changes from blue to yellowish brown, as is the case with chlorine. Ozone therefore conducts itself towards guaiacum resin in precisely the same way as chlorine. It is scarcely necessary to point out that ozone produced by the other two methods, namely, by the electrolysis of water and by the action of the electric discharge on air or moist oxygen, acts in the same way toward guaiacum. Since the bluing of guaiacum undoubtedly depends on the action of oxygen, and since free oxygen does not act on the resinous mass, this element must first be gotten into a state of chemical activity in order to oxidize the guaiacum. This condition seems to be called forth even by sunlight. It is not known, however, whether dry oxygen can act on the water-free resin in the sunlight. It may well be that in the absence of water the guaiacum resin can, as is the case with other organic substances, take up oxygen at ordinary temperatures to a slight extent. Be this as it may, it is nevertheless a fact that the oxygen which is in association with certain other substances has such chemical activity that it can act upon guaiacum, or upon a substance with which it is in combination, at ordinary temperatures. If one believes, as indicated by the older theories, that chlorine, bromine, and iodine are the peroxides of murium, bromium, and iodium, one must believe that there exists in these compounds an equivalent of oxygen which is in this chemically active state, and that it is this oxygen which calls forth the above-described color change in guaiacum resin. If, as we hold, ozone is to be looked upon as a compound of oxygen with water, it is the chemically excited oxygen of this compound which blues the guaiacum. As I have elsewhere pointed out, compounds of certain of the metals act upon guaiacum in the same way as do chlorine and ozone. For example, if one mixes pure lead or manganese peroxide in water with a solution of guaiacum, the latter is instantly colored blue just as if brought into chlorine water. It is remarkable, and so far as I know a new fact, that guaiacum which has been blued in this way loses its color again if it is introduced into an atmosphere containing hydrogen sulfide or sulfur dioxide, or when added to a solution of stannous chloride; under these conditions the oxygen in combination with the guaiacum appears to be again removed. Finally, I have observed that all those substances which color a potassium iodide solution yellow to brownish red, or blue the potassium iodide and starch mixture—that is, all substances which have the power to cause the separation of iodine; for example, chlorine, bromine, ozone, nitrous acid, and the peroxides of manganese, lead, and gold—have also the power to blue the tincture of guaiacum. Conversely, those substances which have the power to remove the yellow color from potassium iodide solutions and the blue color from the potassium iodide and starch mixture—for example, hydrogen sulfide, sulfur dioxide, and stannous chloride—have also the power to destroy the blue color of guaiacum resin.

In a further note on guaiacum resin Schoenbein (371) shows that guaiacum is also blued by the products of the slow combustion of ether, for the reason that an ozonid is produced. Schoenbein (372) next makes use of the bluing of guaiacum as a reaction for indicating the presence of an electric current, he (373) having observed that strips of paper saturated with tincture of guaiacum are colored blue when exposed to the oxygen liberated from water by electrolysis and to the ozone produced by the electric discharge in air or oxygen. In 1847 Schoenbein (374) seems to have published a résumé of his work on the bluing of guaiacum up to that time, mentioning the reduction of the blue compound by hydrogen sulfide and other reducing agents, and the general similarity between guaiacum-blue and the blue iodide of starch. In other still later communications on guaiacum resin (375-376), he calls attention to the considerable number of substances which have the power to blue guaiacum, among which may be mentioned chlorine, bromine, iodine, ozone, certain peroxides such as those of manganese and lead, silver oxide, and acetate, cupric chloride, ferric chloride, mercuric chloride, potassium ferricyanide, the bichromates and permanganates, and even finely divided platinum. He also points out that guaiacum which had been blued by any one of these substances gradually loses its blue color on standing, but that this may be restored by adding fresh quantities of the reagent. This may be repeated a certain number of times, but finally a colorless or brownish product of the resin is obtained which is no longer capable of being blued by ozone or similar substances.

He calls attention to the fact that the blue color of guaiacum is destroyed by phosphorus, the metals, hydrogen sulfide and selenide, sulfurous acid and hyposulfites, ferrous and stannous salts, and by acids and alkalis. He also points out that the majority of those substances capable of bluing guaiacum contain their oxygen in the peculiarly active condition in which this element is met with in ozone. In other words, these substances contain *oxylisirten* or *erregten* oxygen, or can give rise to the same. He points out a number of analogies between guaiacum blue and the iodide of starch, as to color, general methods of formation by the action of oxidizing agents, conduct towards reducing agents, etc., and he reached the conclusion that the blue material resulting from the action of ozone on guaiacum is a compound of ordinary guaiacum with a hydrogen peroxide (ozone), of much the same nature as the loose chemical combination met with in the iodide of starch. He goes on to say further that —

> I need scarcely remark that for those who with De la Rive and Berzelius look upon ozone as nothing but modified oxygen, it is only necessary to assume that guaiacum blue consists of a loose combination of ordinary guaiacum and this uncommon oxygen. In other words, that it possesses the nature of an organic peroxide which contains at least a part of its oxygen in the chemically excited or active condition in which it is met with in hydrogen peroxide, ozone, and manganese dioxide.

Finally, he explained the spontaneous decolorization of guaiacum blue and its ultimate conversion into a substance no longer capable of yielding the blue compound by treatment with ozone or other oxidizing agents, upon the supposition that the chemically active oxygen in loose combination in the blue resin can exist for only a short time as such in this compound and that this form of oxygen at ordinary temperatures, or even at 0°C., acts slowly on the oxidizable constituents of the resin, probably extracting hydrogen and possibly carbon, and thereby altering the original composition of the guaiacum.

Schoenbein (377) next turned his attention to the bluing of guaiacum by those substances contained in the fresh tissues of plants, and in 1848 he gave to the chemical world the results of his first researches in this highly fruitful field of investigation in a communication entitled "On certain chemical reactions of the potato." In this paper he refers to the observations of Planche[a] and Taddei on the bluing of guaiacum by many roots and tubers, and to the fact that the latter appeared to consider air as necessary for the reaction. His own experiments indicate that there is unevenly distributed throughout the potato some substance having the power of bluing guaiacum, the most rapid bluing being produced by the under side of the potato peel and at the points where the "eyes" or sprouts occur. According to Schoenbein, the starch and expressed juice of the potato, however, do not possess this power to the slightest degree, and, upon boiling, all parts of the potato entirely lose their power to blue guaiacum. On the other hand, the freshly cut peel of the potato blues guaiacum as quickly as does manganese dioxide or lead peroxide, and the blue solution shows all of the properties of that obtained by the latter method, fading slowly in the air at ordinary temperature, and rapidly upon boiling being decolorized by hydrogen sulfide and other reducing agents and precipitating a blue resin when added to water. He observed also that the potato, like the other substances having the power to blue the guaiacum resin and tincture has the power of decomposing potassium iodide with the liberation of iodine, the latter reacting with the starch of the potato to form the blue iodide of starch. For these reactions Schoenbein offers two alternate explanations: either that the potato contains a substance analogous to the peroxides, ozone, etc., or that it contains a substance having the power of activating the oxygen of the air, and thus rendering it capable of producing these phenomena.

On the other hand, Nasse and Framm (310) (*see also* Nasse (309)) claim that fresh extracts that have been completely freed of oxygen by the prolonged action of hydrogen or carbon dioxide still give a blue color with guaiacum. Hence, according to these authors, the bluing of guaiacum by plant extracts and ferments, such as diastase and emulsin, is due not to an oxidation of the guaiacum by the oxygen of the air, but to a hydrolysis (*hydroxylirung*) of the guaiacum by the plant extract or ferment. Bertrand (51), however, is of the opinion that the bluing of the guaiacum is due to the combined action of oxygen and laccase, and Kastle and Loevenhart (244) were unable to obtain any evidence of the bluing of guaiacum by the freshly abraded surface of the raw potato in an atmosphere of hydrogen, carbon dioxide, or nitrogen. On the other hand, in air or oxygen, the freshly abraded surface of the potato was always found by these observers to develop a blue color instantly on the application of tincture of guaiacum.

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[a] In all of Schoenbein's original communications on this subject, this author (Planche) is referred to under the name of "Blanche." Thus a good deal of confusion has been introduced into this literature, and this is met with repeatedly, even in recent writings on the subject. Thus this error is repeated by Nasse and Framm in an article entitled "Bemerkungen zur Glykolyse" (Pflüger's Archiv., 1896, vol. 63, p. 207), in which the mechanism of the guaiacum reaction is discussed; and very recently again by Engler and Herzog, in "Zur Erkenntnis der biologischer Oxydationsreaktionen" (Hoppe-Seyler's Zeitschrift für physiologische Chemie, 1909, vol. 59, p. 357). For the benefit of those who may be interested in the historical development of this subject, the writer is glad to be able to correct this error.

OXYGEN-EXCITERS AND OXYGEN-CARRIERS ("SAUERSTOFFERREGERN" AND "SAUERSTOFFTRÄGERN," *Schoenbein*).

In 1855 Schoenbein (378) made a study of the spontaneous bluing of certain fungi. The results of these interesting observations were given to the world in a communication entitled "Ueber die selbst Bläuung einiger Pilze und das Vorkommen von Sauerstofferregern und Sauerstoffträgern in der Pflanzenwelt," and also in a letter to Faraday on "Ozone and ozonic actions in mushrooms." The titles of these two communications give a good idea of their contents. In brief his results were as follows: It had long been recognized that certain varieties of the higher fungi, notably the *Boletus luridus*, have the remarkable property of rapidly turning blue when the head or stem is broken or bruised in any way. Schoenbein now conceived the idea that in such color changes we have to do with phenomena similar to the bluing of guaiacum by the fresh tissue of the potato and other fresh roots. As a matter of fact he found this species of boletus to contain a colorless principle easily soluble in alcohol, and exhibiting the closest analogy to guaiacum, in that all oxidizing agents which blue a tincture of the latter also blue an alcoholic solution of the chromogenic substance of the mushroom, and, further, that all deoxidizing agents which discharge the color of guaiacum blue also discharge the color of the blued fungus extract. From these observations he was led to conclude that the chromogenic principle of the fungus, like guaiacum, is capable of combining with ozonized oxygen, $\overset{\circ}{O}$, whereas it is not affected by ordinary oxygen (O).

The fact that the alcoholic solution of this coloring principle of the Boletus is not spontaneously colored by atmospheric air, but is colored by air in the parenchyma of the fungus, led him to suspect that there exists in the fungus another substance endowed with the property of exalting the chemical power of ordinary oxygen, thereby causing this element in its active form, $\overset{\circ}{O}$, to associate itself with the coloring matter of the fungus. As a matter of fact, he found in the expressed sap of this and other fungi, notably in *Agaricus sanguineus*, an organic matter which has this remarkable power of transforming ordinary oxygen (O), into ozonized oxygen, $\mathring{O}$, and of forming with the latter a compound analogous to lead peroxide ($PbO+\mathring{O}$), which readily gives up this active oxygen, $\mathring{O}$, to a number of easily oxidizable substances, both organic and inorganic, among them guaiacum and the chromogenic substance of *Boletus luridus*. He observed, further, that after having been deprived of its active oxygen, this peculiar compound may be charged with it again by simply passing a current of air through its solution. He then goes on to say that this peculiar substance may well be compared with nitric oxide, which enjoys to a remarkable extent the power of instantaneously transforming inactive oxygen, (O), into active oxygen, $\mathring{O}$, thereby forming a peroxide containing $\mathring{O}$, and from which this peculiar form of oxygen may easily be transferred to a multitude of oxidizable substances. In other words, in addition to the chromogenic substance which it contains and through whose oxidation it ultimately becomes blue, the *Boletus luridus* and other fungi, notably the *Agaricus sanguineus*, contain a substance capable of ozonizing the oxygen of atmospheric air; in other words, they contain a *Sauerstofferreger*, or oxygen-exciter. This substance enters into a loose combination with the ozone thus produced, forming therewith a compound analogous to a peroxide containing active oxygen, which in turn is capable of giving up its active oxygen to guaiacum or to the chromogenic substance contained in the fungus itself, thus turning it blue. In other words, the substance responsible for the bluing of the chromogenic substance contained in the boletus is not only a *Sauerstofferreger* but also a *Sauerstoffträger*, or a true carrier of oxygen.

Schoenbein was also impressed with the instability of these remarkable *Sauerstofferregern* and *Sauerstoffträgern* of the plant world. Thus he points out that on heating to boiling an aqueous solution of the fungus which has the power of deeply bluing a tincture of guaiacum, the solution not only loses this power, but was also found to lose the power of ever again becoming an oxygen-exciter or carrier, no matter how long it was kept in contact with atmospheric air. Finally he goes on to say that these substances which have the power of bringing into activity the inactive oxygen of the air must play an important rôle in the oxidation processes of vegetable materials, and that it is not an unwarrantable supposition that in the animal world as well there exist substances capable of ozonizing atmospheric oxygen at ordinary temperatures, thereby promoting the oxidation of other animal substances, and in a later article (380, 386) on the conduct of oil of bitter almonds to oxygen, in which he proved that this compound belongs to the class of ozonizing substances, he says that new observations have made it in a high degree probable that there exist in the blood of animals substances having the power to change the respired inactive oxygen in a similar way to that accomplished by phosphorus and oil of bitter almonds—that is, substances which render the oxygen active, whereby the oxidation phenomena observed in the life cycle are brought about. He adds, further, that without the presence of such substances as convert (O) into $\mathring{O}$ the animal would be suffocated in the midst of an ocean of the purest, but inactive, oxygen as quickly as it would be in a vacuum.

Schoenbein's remarkable observations in this field, however, did not end here. From his extended investigations on ozone and hydrogen peroxide he had reached the conclusions that oxygen can exist in three forms, viz, common oxygen, which he represented by O or (O), ozone, Θ or (Θ), and antozone, $\oplus$ or $(\oplus)$; and that deriving from these three modifications of oxygen, respectively, we have three classes of oxides, viz, (1) ordinary oxides, such as water; (2) ozonides, which included most if not all of the peroxides of the heavy metals, such as manganese dioxide and lead peroxide, and (3) antozonides, like hydrogen peroxide and barium dioxide

O	Θ	$\oplus$
HO (old formula),	$MnO + \Theta$,	$HO + \oplus$,
CaO,	$PbO + \Theta$,	$BaO + \oplus$.

Of these several forms of oxygen, the ozonic modification was the most active. He had observed, further, that just as the common oxygen is transformed into ozone through the influence of ozonizers, such as phosphorus, platinum, etc., so the oxygen of the antozonides $\oplus$ may be transformed into the ozonic modification, Θ, by the action of certain substances such as platinum or lead salts, etc., and that finally as a result of the neutralization of their electrically opposite potentials, an ozonide reacts with an antozonide with the production of common oxygen. Thus ozone and hydrogen peroxide give rise to common oxygen and water:

$$\Theta + HO + \oplus = HO + 2O.$$

He found, for example, that guaiacum blue results from the action of finely divided platinum on tincture of guaiacum and air or hydrogen peroxide, whereas neither of these substances can effect this alteration in the color of the guaiacum, or can do so only with extreme slowness, when used alone. It had been previously observed by Thenard that hydrogen peroxide is decomposed by finely divided platinum into water and common oxygen. All of these facts found a simple explanation in terms of Schoenbein's theory. According to this theory, neither common oxygen nor antozone nor an antozonide can blue guaiacum, for the reason that the resin only combines with the ozonic form of oxygen, Θ. On the other hand, ozone or any ozonide can accomplish this change for the reason that any of these substances can supply Θ, and platinum can accomplish this oxydation by common oxygen or hydrogen peroxide for the reason that it can transform common oxygen or the ⊕ of an antozonide into ozone, Θ. This theory also enabled one to understand the catalytic decomposition of hydrogen peroxide by platinum into water and common oxygen, since the ozone resulting from the action of the platinum on the antozonide would react with another portion of the antozonide, giving rise to water and common oxygen (382):

$$\mathrm{Pt}+\mathrm{HO}+\oplus=\mathrm{Pt}+\mathrm{HO}+\Theta,$$

and

$$\mathrm{HO}+\Theta+\mathrm{HO}+\oplus=2\mathrm{HO}+2\mathrm{O}.$$[a]

In 1857 Schoenbein published a paper on "Chemical contact actions" (379), in which he discusses the action of various oxygen-carriers and oxidizing agents on guaiacum, and states that blood corpuscles separated from fibrin and serum and dissolved in water give the guaiacum reaction strongly in the presence of hydrogen peroxide, as does also the gluten of wheat, but that both are inactive in the absence of the peroxide. He regards this oxygen-carrying power of blood corpuscles as important to respiration. In this article he also gives the results of experiments with platinum and guaiacum. Three years later he (381) called attention to the fact that iron salts act toward a mixture of guaiacum and hydrogen peroxide in much the same way as do blood corpuscles and that the activity of the blood corpuscles toward guaiacum is proportional to their content of hemoglobin, and that it therefore seemed not improbable that the guaiacum reaction of blood is dependent on the iron content of the pigments. This, he says, is a matter of importance to physiological chemists.

In view of his previous work on the presence of oxygen exciters and oxygen carriers in certain of the higher fungi and in the potato, it was only natural that it should have occurred to Schoerbein, especially in the light of the earlier observations of Planche, that substances capable of bluing a mixture of hydrogen peroxide and guaiacum and of decomposing hydrogen peroxide into water and common oxygen should be widely distributed in the plant and animal kingdom. The results of his investigations on the decomposition of hydrogen peroxide by plant and animal extracts and ferments and on the bluing of guaiacum by hydrogen peroxide through the agency of organic materials were first presented in a paper entitled "Ueber die katalytische Wirksamkeit organische Materien und deren Verbreitung in der Pflanzen- und Thierwelt" (386) in 1863. He calls attention to the fact that in 1863 the group of phenomena called by Berzelius "catalytic actions" were as yet but very imperfectly understood, especially such as are concerned with changes in the composition of organic compounds brought about by the action of ferments, such as the alcoholic fermentation and the conversion of starch into sugar through the action of diastase. He was therefore inclined to the opinion that the decomposition of hydrogen peroxide into water and common oxygen by the action of platinum and similar metals may be regarded as the prototype of all catalytic phenomena. Indeed, as he points out in a later part of this article, Berzelius had already compared the decomposition of hydrogen peroxide by the noble metals to the fermentation of grape sugar by yeast. It was therefore with the view of throwing still further light on the causes of catalysis in general that these studies on the decomposition of hydrogen peroxide by substances of animal and vegetable origin were first undertaken. He observed that platinum and the noble metals generally rapidly blue a tincture of guaiacum containing hydrogen peroxide, and that a certain amount of the hydrogen peroxide present is decomposed into water and common oxygen. It follows from this that the power of these substances to catalyze hydrogen peroxide is so intimately associated with their power to blue the tincture of guaiacum containing hydrogen peroxide that both properties are referable to the same cause, and it was for this reason that a tincture of guaiacum containing small amounts of hydrogen peroxide proved to be a valuable reagent in the investigation of these phenomena. By means of it he proved that these catalytic agents have the widest possible distribution in vegetable and animal tissues. Among the substances tested with the guaiacum hydrogen peroxide reagent were a number of the common enzymes, such as diastase, emulsin, myrosin, rennin, etc., yeast, gluten, and over 100 species of plants, including certain fungi and molds, and also a number of seeds and substances of animal origin, such as fibrin, red blood corpuscles, and saliva. Without exception, all of these substances were found by him to actively decompose hydrogen peroxide, and with only two exceptions, yeast and rennin, all of them likewise gave rise to the production of guaiacum blue. Schoenbein (387) therefore concluded that the power to decompose hydrogen peroxide and to blue guaiacum thereby is intimately associated with the specific activity of the unorganized ferments and with such vital phenomena as the sprouting of seeds, etc., inasmuch as all of these biologic properties are lost by heating to 100° C., by exposure to hydrogen sulphide, and also, as shown in a later paper (388), by exposure to hydrocyanic acid.

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[a] A good discussion of Schoenbein's ozone and antozone theory is to be found in his paper on the "Conduct of blood toward oxygen" (384), a translation of which is given in the Medical Press and Circular, June 20 and July 4, 1866, and in Day's paper (15) on "Polarized or allotropic oxygen," in the Australian Medical Journal, vol. 12, 1867, pp. 141–149.

In still another communication in the year 1863, on "The conduct of blood toward oxygen" (384), he points out that ozone and hydrogen peroxide are formed simultaneously in a large number of slow oxidations, such as the oxidation of phosphorus in a moist atmosphere. In a large number of cases, however, only hydrogen peroxide is met with as the by-product of the oxidation, as, for example, in the oxidation of metallic substances, pyrogallic acid, etc.; and in still another large group of cases neither ozone nor hydrogen peroxide are produced, for the reason, probably, that the latter oxidations are accomplished by common oxygen without activation in any way. He then goes on to say that the view has long been prevalent that in all oxidations resulting in the formation of ozone or hydrogen peroxide the conversion of common inactive oxygen, (O), into ⊕ or Θ takes place. The former, ⊕, then unites with water to form hydrogen peroxide, HO + ⊕, whereas the latter, Θ, is either consumed at once in the oxidation or appears as ozone. Indeed, according to Schoenbeir, Θ is ozone. He therefore arrived at the conclusion that the neutral oxygen which is taken into the body during respiration must obviously suffer such a change of state before it can accomplish the oxidations ordinarily taking place in the animal organism, and since both ozone and hydrogen peroxide result from the oxidation of many substances, he expected to find these substances in the blood of animals. As a matter of fact, however, although he employed the most delicate reagents in testing for these substances in the blood, he was unable to find a trace of either ozone or hydrogen peroxide therein. In this connection he points out, however, that Hiss had shown that ozone readily oxidizes the albumin and fibrin of blood and also the blood corpuscles, and that long ago Thenard had shown that fibrin readily decomposes hydrogen peroxide into water and common oxygen, and he himself had shown that blood corpuscles obtained from defibrinated blood also rapidly decompose hydrogen peroxide, and that a solution of defibrinated blood of such dilution as to no longer exhibit the characteristic color of blood rapidly blues tincture of guaiacum containing hydrogen peroxide. He concludes, therefore, that while the common oxygen consumed in respiration is resolved into ⊕ and Θ, the latter, Θ, is at once consumed in oxidations, and that the former, ⊕, unites with water to form hydrogen peroxide (HO + ⊕); which, however, is rapidly decomposed by the red blood corpuscles into water and common oxygen. This again enters into the ordinary cycle of changes met with in the absorption and utilization of the respired oxygen, viz, into the formation of ⊕ and Θ, so that ultimately all of the oxygen would be gotten into a form available for oxidations, viz, Θ.

It should likewise be borne in mind that the blood corpuscles not only have the power of decomposing hydrogen peroxide into water and common oxygen, but also of ozonizing the oxygen, ⊕, which it contains, after the manner of a lead salt, as indicated by the fact that a solution containing the peroxide and red blood corpuscles rapidly blues a tincture of guaiacum. In the light of these facts, therefore, one can readily understand, he says, why the red blood corpuscles have the power of rapidly decomposing hydrogen peroxide, nor should one be inclined to look upon this as a useless property, since the blood corpuscles also have the power of ozonizing a part of the oxygen of the peroxide after the manner of platinum or a lead salt. According to Schoenbein, therefore, the red blood corpuscles, by virtue of their activating and catalyzing properties, play an important rôle in all processes of oxidation occurring in the organism of the higher animals, inasmuch as it is scarcely conceivable that their oxidizing power should be confined entirely to guaiacum resin or pyrogallic acid.

So far as the historical development of the subject is concerned, the work on the oxidases is approximately divisible into three periods. The first period begins with the earlier observations by Planche, Taddei, and Schoenbein, on the bluing of guaiacum. The second period has to deal with the work of Moritz Traube. The third period begins with the work of Yoshida and Bertrand on laccase, and includes all recent investigations on the subject. Obviously, as is the case with all things historically considered, the work of each of these periods overlaps somewhat the work of the other periods.

To review briefly the salient facts brought to light during the first period, we note that Wollaston was the first to call specific attention to the bluing of guaiacum and to the part played by air and light in effecting this change. William Brande also ascribed this change of color to oxygen, and succeeded in showing that pure oxygen gas effects the change somewhat more rapidly than atmospheric air. Then followed the earlier observations of Planche, that guaiacum is rapidly blued by the vapors of nitric acid and also by the fresh root of the horse-radish. This last-mentioned observation, made in 1809, marks the first recognition of a change brought about by an oxidizing ferment, although the ferment-like nature of the substance causing the change was not definitely recognized until many years afterwards. Other French observers noted a bluing of guaiacum by gums, such as gum arabic, and also by saliva. Taddei showed that under the influence of air and moisture the gluten of wheat flour blues guaiacum, and Planche showed that this reaction is characteristic of the roots of a large number of plants, and also of fresh milk, and, further, that the power to blue guaiacum on the part of these substances was lost on boiling. Thus, without fully appreciating its significance, this observer discovered one of the most characteristic properties of the oxidases as a class, viz, the loss of oxidizing power on boiling. Planche however failed to recognize the part played by atmospheric oxygen in these changes, and attributed the bluing of guaiacum by plant tissues to a peculiar unstable principle which he called *cyanogene.*

After the work of Priestley and Lavoisier, the greatest impetus to a better understanding of the processes of oxidation was given by the discovery of ozone by Schoenbein in 1840. We have seen that this observer recognized this active variety of oxygen among the products of slow oxidation, and that he explained the phenomenon of oxygen carrying as due to the production of ozone or of an ozonide. He greatly extended our knowledge of the bluing of guaiacum, and also the earlier observations of Planche on the presence of oxygen exciters in plants and animals, and he accounted for the remarkable oxidizing activity of such substances on the supposition that they, in common with platinum, nitric oxide, and other oxygen carriers, ozonize the air, combining with the ozone thus produced to form an active ozonide, which in turn can give up its oxygen to other less readily oxidizable substances. These were his *Sauerstofferregern* and *Sauerstoffträgern.* They are what we to-day term the oxidases, or oxidizing ferments. Through the action of these substances on certain chromogens he was able to explain the changes of color observed in the growth and decay of certain species of the higher fungi, and similar changes occurring in certain fruits, such as take place in the apple when peeled or crushed. He also recognized the wide distribution of substances capable of decomposing or catalyzing hydrogen peroxide and of greatly activating the latent oxidizing powers of this substance; and all of these facts he was able to explain more or less satisfactorily in terms of his theory respecting the relation existing between common oxygen, ozone, and antozone, according to which ozone and antozone were oppositely electrified; that under the influence of various substances, like platinum, etc., common oxygen and antozone or an antozonide could be transformed into ozone or an ozonide, and finally that ozone could react with an antozonide, such as hydrogen peroxide, to form common oxygen. In this connection it may be observed that no one can read the writings of Schoenbein without being impressed with the great wealth of his observations, and his theory regarding the nature of ozone will always serve to remind the thoughtful student of chemistry how clearly and apparently how accurately a great variety of phenomena can ofttimes be explained by an hypothesis altogether erroneous in its premises.

It is evident, therefore, that during the first period, covering approximately the first six decades of the nineteenth century, the presence of oxygen activators and carriers in the plant and animal world had been recognized, and the more important characteristics of the oxidases and peroxidases had been discovered, so far as we have an accurate knowledge of these properties to-day, and yet the terms "oxidase" and "oxidizing ferment" had not up to this time been introduced into the science, the nearest approach to them being the use of the terms *Sauerstofferreger* and *Sauerstoffträger* employed in the later writings of Schoenbein, and the recognition of the fact by this author that the power to activate oxygen and hydrogen peroxide is more or less intimately associated with the zymotic activity of the unorganized ferments and with vital phenomena generally. He accounted for all of these phenomena on the supposition that by means of various substances and under various influences the oxygen of the air becomes ozonized.

In the meantime other chemists and physicists (see Soret (405), Andrews and Tait (12), Brodie (102), Odling (313)) had gradually come to distinguish more clearly between the three forms of oxygen, viz. ordinary oxygen O_2, ozone O_3, and active (atomic) oxygen, O (antozone). The result has been that Schoenbein's original views regarding the manner in which the *Sauerstofferregern* and *Sauerstoffträgern* effect the activation of ordinary atmospheric oxygen, and the transfer of oxygen to other less readily oxidizable substances thereby, have been considerably modified and our knowledge of what we know as the oxidizing ferments considerably extended.

OXIDIZING FERMENTS ("VERWESUNGSFERMENTE" AND "OXYDATIONSFERMENTE," *Traube*).

During the second period of the development of this subject our knowledge of processes of slow oxidation and oxygen-activation, as well as of fermentation in general, was considerably extended through the labors of Moritz Traube. It remained for Traube to introduce into the science the term "oxidizing ferment" (*Oxydationsferment*) as a generic term for the unstable *Sauerstofferreger* and *Sauerstoffträger* of Schoenbein, which were found by Planche and Schoenbein to be so widely distributed among plants and animals.

In 1858 Traube (125) gave to the world his "Theory of Fermentation" (Theorie der Fermentwirkungen, von Moritz Traube, Berlin, Ferd. Dümmlers Verlagsbuchhandlung, 1858). A brief outline of this theory is also given in a paper published in Poggendorf's Annalen (126), and later he developed his ideas in a series of articles in the Berichte der Deutschen Chemischen Gesellschaft. In paragraph 175, page 61, of Traube's "Theorie" it is stated that an essential distinction between putrefactive and vital ferments—that is, those present in the living organism—does not appear to exist.

In this treatise and in his later papers he developed the idea that the ferments are not bodies in a state of decomposition which impart decomposition to other substances, as had been supposed by Liebig, but are, in all probability, chemical compounds originating from proteids which, while not as yet isolated in pure condition, have, without doubt, as have other compounds, a definite chemical composition, and which, through the exercise of definite chemical affinities, produce alterations in other compounds. He held also that Schwann's hypothesis, as subsequently developed by Pasteur, namely, that fermentation is the result of the action of the life phases of lower organisms is insufficient, and that rather the converse of this hypothesis is really correct, namely, that the ferments are the causes of the important bio-chemical processes and are not confined to the lower organisms, but are present also in the higher forms of life. (*See* Traube's "Theorie," supra, paragraph 175, p. 61.) In this connection he pointed out that the ferments are produced at ordinary or slightly elevated temperatures from the decomposition of protein substances by the action of water, probably under the influence of oxygen. Thus fresh meal prepared from dry grain contains no diastase. If, however, the grain acts upon water in a limited supply of air, chemical changes occur and diastase is generated. (Par. 172, p. 60, Traube's "Theorie.") He also pointed out that in many instances at least, the ferments are ideal catalysts, acting as chemical go-betweens between free or combined oxygen and the fermentable substances. Thus the blood is an ideal carrier of free oxygen, in that in the lungs it becomes saturated with oxygen, which is used for the purpose of direct oxidation in the capillaries, from which it is returned to the lungs to recombine with oxygen.

He was led to believe further, as a logical corollary of his theory, that various organic and inorganic substances other than those elaborated in the living cell may under certain conditions function as ferments. That such is the case is shown by the fact that platinum is a carrier of oxygen; that is, it may exert the same sort of action as the *Verwesungsfermente* (oxidizing ferments), and that in indigo-sulphuric acid we have a substance capable of exhibiting changes altogether analogous to certain kinds of fermentation. Later he (128) showed in this connection that platinum black at 150° to 160° C. decomposes solutions of sugar with the production of carbon dioxide and of a light oil, which gives the iodoform reaction.

Briefly put, Traube's theory of fermentation is based upon two distinct chemical propositions; first, that the ferments are definite chemical compounds elaborated from proteid as a result of the combined action of heat, water, and oxygen, and present not only in the lower organisms but also in the tissues of the higher forms, where they are responsible for bio-chemical processes; second, that the ferments are powerful reducing agents and oxygen carriers, capable in their capacity of chemical go-betweens (*Vermittler*) of effecting the transfer not only of free oxygen to easily oxidizable substances but also the transfer of combined oxygen from one compound to another.

He was led to divide ferments into three classes:

(*a*) *Verwesungsfermente*, those which combine loosely with free oxygen, forming unstable compounds which give up their oxygen to other less readily oxidizable substances.

(*b*) *Reductionsfermente*, those which combine with the oxygen of water, the hydrogen going to effect the reduction of the passive body.

(*c*) *Hochstes Faulnissfermente*, those which cause putrefactions in which hydrogen is set free.

He was led to believe further that all of these ferments have the power of carrying to other substances the oxygen with which they have combined, thereby becoming reduced and gotten into the condition whereby they can again combine with fresh quantities of oxygen and again carry it to oxidizable substances. In this way they are able to carry free and combined oxygen in practically unlimited quantities to other substances, and in that way bring about gradual decay (*Verwesung*) and fermentation proper (*Gährung*). (Traube, [425, 426].) In the one case (*Verwesung*) the oxygen required for the oxidation comes from the air; in the other case (*Gährung*) it comes from the water. Hence in all true fermentations water acts not merely as a solvent, but actively participates in the process. In this discussion, however, we are primarily concerned only with his first class of ferments, viz, the *Verwesungsfermente*. These he divided into two groups: *Vitale*, those met with in the higher life forms, and *faulige*, putrefactive, those concerned in the change and decay of dead material. In the first class he placed the guaiacum bluing ferment of the potato and the red coloring matter of blood. In the second class he placed the ferments concerned in the transformation of alcohol into acetic acid and the nitrogenous compounds of dung into nitrates. As nearly as I have been able to determine, the term "*Verwesung*," as employed by Traube in this connection, seems to have been borrowed from the writings of Liebig, and in terms of the present nomenclature employed for processes in which the oxidizing ferments are concerned, it is practically untranslatable. As ordinarily employed, it signifies putrefaction or decay. In reality, as employed by Liebig, it referred primarily to the spontaneous decay of vegetable matter, whereby this sort of material is ultimately converted into brown mold or humus-like substances. Other English writers have encountered this difficulty in dealing with this word and its derivatives. Thus in translating Liebig's Agricultural Chemistry Gregory was forced to coin the word *eremacausis* (from the Greek, meaning "slow-burning") as the equivalent of *Verwesung*, the introduction of this term being made with Liebig's consent. In his earlier writings on the subject Traube made constant use of the term *Verwesungsfermente* as signifying the oxidizing ferments, to the exclusion of practically all other names for these substances. In Paragraph 132, page 49, of his "Theorie," it will be seen, however, that he employs the term "*Sauerstoffübertragenden Fermente*" (oxygen-carrying ferments) for the vegetable oxygen carriers described by Planche and Schoenbein, and later, in 1874, in an article on the conduct of yeast in oxygen-free media ([429]), referring to his earlier work, he says:

> I have given several examples to show that there are substances (in animal and vegetable tissues) which, like platinum, nitric oxide, and indigo-sulphuric acid, carry free oxygen to other substances and thereby accomplish their oxidation. (*Sauerstoffübertrager, oxydationsfermente.*)

It was in his later writings ([430]), however, especially in his controversy with Hoppe-Seyler that he insists upon the use of the term *Oxydationsfermente* (oxidizing ferments) as preferable to the term *Verwesungsfermente*, which latter term he had previously employed as signifying those ferments which possess the power of taking up free oxygen and carrying it to other passive substances, thereby accomplishing the oxidation of the latter.

As the result of their action, the oxidizing ferments suffer no chemical alteration, playing merely the part of go-betweens in oxidation, in that they alternately combine with oxygen and give it up to other substances. Obviously, according to Traube, free oxygen is essential to their action. In this connection his earlier observations on the oxidizing ferments of the potato are of interest. He showed that if thinly cut potato peel is rubbed up in a mortar with a small amount of distilled water, a solution is obtained which blues guaiacum intensely. If the blue solution thus obtained be allowed to stand in a reagent glass it bleaches, except on the upper surface where it is in contact with the air. On shaking with air, however, the whole solution again acquires a deep blue color, which on standing again bleaches. This alternate bluing of the solution by shaking with air and subsequent decolorization of the lower layer of the solution upon standing was observed upon one and the same solution for a period of fourteen days, indicating that the potato peel contained a true oxygen carrier through the instrumentality of which the oxygen of the air was transferred first to the guaiacum and subsequently to other substances. He also showed that the potato loses its power to blue guaiacum at 80° C., and that the unstable oxygen carrier which it contains is soluble in dilute alcohol. He points out that a body of such remarkable chemical conduct and of such wide distribution in the plant kingdom must play a significant rôle in the growth of vegetation. Similarly, he also recognized the red coloring matter of the blood as a powerful oxygen carrier, and was led to regard muscular activity as a respiratory act in which the muscle fiber functions as an oxidizing ferment in that it unites with the oxygen supplied by the blood to form an unstable compound, which in turn gives up its oxygen to other less readily oxidizable compounds contained in the muscle fluids both in rest and active work, resulting finally in the production of animal heat and work. In other words, muscular activity and the production of animal heat were accomplished, like all *Verwesungsprocessen*, through the action of *Verwesungsfermente* (oxidizing ferments) on the free oxygen of the air. (Traube (127-130).)

In plants, under the influence of oxidizing ferments as go-betweens, oxygen accomplishes the conversion of soluble carbohydrates into cellulose, and in animals it converts soluble proteids into ferments. He goes on to say further that the lessons to be learned concerning oxygen are only limited by those to be learned of organic life itself.

OXIDASES (**Laccase,** *Bertrand*).

A new impulse to the study of the oxidases was given by the beautiful researches of Yoshida, and later by those of Bertrand, on the changes occurring in the sap of the lac tree, *Rhus vernicifera*, and allied species. It had long been known to the Japanese that the milk-like sap of the lac tree dries and hardens on exposure to the air and turns brown and finally black, thereby yielding a lustrous, translucent varnish, which is highly prized on account of its luster and stability. In 1883 Yoshida (167) for the first time undertook an accurate investigation of these interesting changes. He found that the lac tree yields a strongly corrosive sap, which he called "urushi;" this he found to consist essentially of four substances, viz., water, urushic acid, $C_{14}H_{18}O_2$, gum, and a peculiar diastatic matter which possesses the power of transforming the urushic acid into oxy-urushic acid, $C_{14}H_{18}O_3$, through the action of the oxygen of the air, in the presence of moisture. He found that this nitrogenous compound loses this property when heated to 63° C. He was therefore led to conclude that in this substance we have an enzyme which has the power of carrying oxygen to the urushic acid, thereby transforming it into oxy-urushic acid which is the basis of lac varnish. He also showed that urushic acid can be transformed into oxy-urushic acid by chromic acid.

Eleven years later a more exhaustive investigation of the changes occurring in the sap of the lac tree was undertaken by the French chemist Bertrand (18-19), with the result that Yoshida's previous results were confirmed. According to Bertrand, the changes observed when the fresh juice of the lac tree is allowed to stand in the air are brought about by the oxygen of the air under the influence of an oxidizing ferment (*diastase oxydante*) to which he gave the name of laccase. After boiling laccase loses the power of bringing about these changes. Hence he regards laccase as the provocative agent of the oxidation, and found it not only in the fresh juice of the lac tree but also in gum arabic and gum senegal. According to Bertrand, the oxidizable substance present in the juice of the lac tree and which

Yoshida had called urushic acid, shows many resemblances to the polyatomic phenols. He therefore called this substance "laccol," and in view of this analogy he was led to study the conduct of laccase toward a number of the polyatomic phenols and related compounds with the result that he found many of these to be oxidized under these conditions. Thus, hydroquinon is converted by laccase into quinon and quinhydron, with the absorption of oxygen (56). Thus, a 1 per cent solution of hydroquinon alone or with boiled laccase does not absorb oxygen from the air nor is it altered in any way. In the presence of a small amount of laccase, however, such a solution absorbed 25.4 c. c. of oxygen in three hours. With tannin and pyrogallol the oxidation was always attended with the disengagement of considerable amounts of carbon dioxide. Thus, in two experiments oxygen was absorbed and carbon dioxide disengaged in the following amounts:

	Oxygen Absorbed.	Carbon dioxide disengaged.
(1)	23.3 c. c.	13.7 c. c.
(2)	29.8 c. c.	16.4 c. c.

He also found guaiacum to be a useful reagent for laccase, and by means of it he was able to detect the enzyme in many plants, such as potatoes, apples, peas, quinces, lucerne, clover, asparagus, turnips, chestnuts, and various rhizomes (57).

Bertrand and Bourquelot (63), by the use of guaiacum, found laccase in a large number of species of mushrooms. Out of 18 species examined at this time only two, *Polyporus sulfureus* Bull. and *Squamosus* Huds., were found not to give the guaiacum reaction. Hence laccase is not confined to the chlorophyllous plants, but occurs in the nonchlorophyllous plants also.

The oxidations occurring in the juice of the apple, observed in cider-making, were also studied about this time by Lindet (271). This author also claims to have reached the conclusion, as early as 1893, that a soluble ferment governs this oxidation, and in a later article he (272) concludes that the changes of color occurring in fresh cider are due to the oxidation of tannin by a ferment, of the type of laccase, contained in the tissues of the apple.

In the course of his investigations Bertrand found that laccase always contains small amounts of manganese and that its oxidizing power is proportional to the amount of manganese present; and further that oxidations accomplished by laccase are greatly accelerated by small amounts of manganese salts and that no other metal is capable of accelerating the oxidation brought about by laccase.

In the course of these investigations the old observations by Schoenbein (378) that various fungi become colored in the air was also confirmed by Bertrand and Bourquelot (64). They arrived at the notion that the change of color is brought about by an oxidizing ferment which they held to be identical with laccase. The chromogen of the blue substance was later isolated by Bertrand and called boletol (60). On the other hand it was known that certain fungi become red and then black (not blue) on exposure to the air. In such fungi Bertrand and Bourquelot (64) found tyrosin. Now, since this compound is not oxidized by laccase but is oxidized by the fresh extract of those fungi in which it occurs, they ascribed its oxidation to a second oxidizing ferment which they called "tyrosinase." Bourquelot found tyrosinase in many plants, including the potato and sugar beet.

From these observations Bertrand was led to regard laccase as one of a group or class of oxidizing ferments to which he gave the general name of "oxydases." Thus in an article on the relationships existing between the constitution of certain organic compounds and their oxidizability under the influence of laccase he (52) says in footnote 2, on page 793:

> I propose to apply the generic term *oxydases* to soluble oxidizing ferments in order to distinguish them from the true diastases (ferments) which produce double decomposition with the fixation of water.

To Bertrand, therefore, we owe the introduction into the science of the term *oxydase*.

Boutroux (92) has objected to the use of the term *oxydase* as proposed by Bertrand for such oxygen carriers as laccase and that contained in bran, on the ground that the termination *-ase* has been employed exclusively to indicate a hydrolytic ferment, and for the further reason that the formation of such a word as *oxydase* is contrary to the general rule governing the nomenclature of enzymes, according to which the stem of the word thus employed designates the substances upon which the enzyme exerts its action: thus *maltase* signifies the ferment which hydrolyzes maltose. On the other hand it should be borne in mind that this nomenclature for the unorganized ferments has never been rigorously applied, as indicated by the use of the terms *invertase* and *diastase*. Furthermore, the term *oxydase* was well chosen, since these ferments all act upon oxygen or a peroxide, and such terms as *laccase* and *tyrosinase*, etc., serve to differentiate the oxydases from one another and likewise indicate the substances upon which the oxygen acts.

As is the case with other soluble ferments, the oxidases have probably never been obtained in a condition of purity. Indeed, we have no reliable criteria whereby to judge of their purity, and hence but little is known regarding their composition. From the fact that laccase always contains manganese, and that a salt of this metal greatly increases the activity of the oxidase, Bertrand reached the conclusion that laccase consists of manganese in combination with a proteid radicle. The former functions in the capacity of a co-ferment, whereas to the latter the laccase owes its zymotic characteristics, such as destructibility by heat, poisons, etc.

According to Portier (³³⁰), the following properties are characteristic of an oxidase:

(1) The power to oxidize certain substances in the presence of free oxygen, gaseous or dissolved.

(2) The power to cause the absorption of oxygen during oxidation.

(3) Destruction by heat.

(4) Nondialyzability.

According to Bourquelot (⁸⁴), the oxidases belong to the class of enzymes and possess the following characteristics common to enzymes in general:

(1) Catalytic power, viz., power to effect the transformation of an indefinite amount of material by means of an infinitesimal quantity of the ferment.

(2) Like other ferments, their activity is subject to regular and constant influence by heat, increasing in oxidizing power with rise in temperature to 42°–45° C. (optimum temperature), then falling off in activity with further rise to 60°–70° C., and finally completely destroyed at 100° C.

(3) Insolubility in alcohol.

(4) Solubility in water, even after precipitation by alcohol and desiccation.

(5) Adsorption by precipitates (colloids).

(6) Absence of power to dialyze.

In addition to these general characteristics, they possess, according to this author, the following properties properly belonging to the oxidizing ferments:

(1) Power of accomplishing oxidation by means of gaseous or dissolved oxygen.

(2) The accompaniment of the oxidations accomplished by them with a notable absorption of oxygen.

Duclaux (¹⁵⁰, vol. 2, p. 565), has defined oxidases as substances which at ordinary temperatures and under physiologic conditions, carry oxygen rapidly to substances upon which, without the intervention of oxidases, ordinary oxygen would act very slowly.

The oxidases are very readily soluble in water; they are also readily soluble in glycerin, but in other organic solvents thus far investigated they seem to be insoluble. Thus I (²³⁸) have found the oxidase of *Lepiota americana* to be very soluble in water, glycerin, 40 per cent formaldehyde, and mixtures containing water and alcohol, but insoluble in ethyl, amyl, and allyl alcohols. This fungus also gave up its oxidase to a preservative solution containing equal quantities of water and alcohol to which a small amount of formaldehyde had been added. Some observations seemed to show that it was soluble in toluene to some extent. It has also been my own experience that aqueous solutions of the oxidases are very unstable even in the presence of mild antiseptics. Thus aqueous extracts of the potato soon lose their power to blue guaiacum, even when preserved under antiseptic conditions. Bourquelot observed that an aqueous extract of *Russula delica* when preserved with chloroform first loses its power to oxidize tyrosin, then guaiacol, and finally, after eight weeks, its power to oxidize guaiacum. I have found aqueous extracts of *Lepiota americana* to oxidize guaiacum at the end of eighteen weeks. Bertrand found tyrosinase to be very unstable. On the other hand, according to Bourquelot (75), aqueous solutions of tyrosinase to which chloroform had been added were found to be active at the end of two or three months.

In glycerin the oxidizing ferments are much more stable. Thus Gessard (181) has found that a solution of tyrosinase in glycerin, obtained from mushrooms by Bourquelot's method, is stable, and in his work he employed such an extract which had retained its activity for ten months. Bourquelot (81) found that glycerin extracts of *Lactarius velutinus* retained their oxidizing properties for a year or longer. In the course of my own work on this subject I have found that glycerin extracts of *Lactarius piperatus* and *Lactarius volumen* prepared in the summer of 1905, both of which, in the fresh state, were found to be quite active toward guaiacum and tyrosin, were still active toward these substances in the summer of 1909.

As a general thing, the oxidizing power of an oxidase is not confined to one particular oxidizable substance. Thus laccase has been found to oxidize not only laccol, the oxidizable principle of the juice of the lac tree, but also to oxidize guaiacum, guaiacol, hydroquinone, phenolphthalin, and a large number of phenols and aromatic amino compounds. (See pp. 59–61.) So also tyrosinase oxidizes not only tyrosin but also a large number of related compounds. (See pp. 81–84.) In the same way an oxidase which will oxidize benzaldehyde to benzoic acid will also oxidize salicylic aldehyde, benzyl alcohol, and related substances. On the other hand laccase will not oxidize tyrosin, nor will tyrosinase oxidize guaiacum or phenolphthalin. So in the same way many animal tissues which have been found to oxidize salicylic aldehyde are without effect on guaiacum, hydroquinone, and tyrosin. While thus not absolutely confined in their action to any one particular oxidizable substance, the oxidases exhibit a certain degree of specificity in the sense that they are more or less limited in their action to certain groups of substances which are more or less closely related chemically.[a]

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[a] From his studies on the spontaneous oxidation of the sugars, Mathews (280) concludes that two distinct groups of substances have been confused under the name of *oxidases*; one group, the oxidases proper, activates oxygen more or less generally toward all oxidizable substances, while the other group of ferments, which are more specific in their action, activates certain oxidation processes, such as the oxidation of sugars, by causing a dissociation of the sugar molecule. For the latter he proposes the name *metabolase*, since they hasten metabolism. According to this author the failure on the part of the organism to burn glucose under certain conditions is probably not due to the absence of oxidases, but to the loss of its power to dissociate glucose into easily oxidizable substances.

CLASSIFICATION OF THE OXIDASES.

Various attempts have been made from time to time by different observers to effect a classification of the oxidases. None of these are altogether satisfactory, but are probably as good as can be made in the present state of our knowledge of these substances.

According to Grüss (200) there are three classes of oxidases in the higher plants, namely α-oxidases, β-oxidases, and γ-oxidases. The α-oxidases (1) act directly upon guaiacum and tetramethyl-para-phenylene diamin with the fixation of oxygen; (2) they are soluble in glycerine and are partially precipitated from their solutions by acetate of lead, and are easily destroyed by alcohol; (3) they are found in the parenchymatous tissue of the potato and in most dicotyledenous plants. The β-oxidases (1) activate the oxygen of peroxides, and hence they only blue guaiacum or act upon tetramethyl-para-phenylene diamin in the presence of hydrogen peroxide or a similar substance; (2) they are soluble in glycerine and are precipitated from their solutions by alcohol and ether without being destroyed; (3) they have been found in the resting, mature tubers of the potato. The γ-oxidases (1) also activate the oxygen of peroxides, and hence only blue guaiacum or act upon tetramethyl-para-phenylene diamin in the presence of hydrogen peroxide; (2) they are not destroyed even by boiling alcohol, and in order to demonstrate their presence the tissue must be boiled in alcohol for a short time before adding the hydrogen peroxide and oxidase reagent; (3) they have been found in old wounds in plants such as the potato, in the diastase of the barley grain, in association with cytase, in the leptome of growing roots, in *Astragallus glycophylloides*, etc.

Grüss has also classified certain of the vegetable oxidases according to differences in conduct toward certain oxidase reagents. Thus he observed that certain oxidases can act upon tetramethyl-para-phenylene diamin, but not upon guaiacum, whereas other vegetable tissues are capable of oxidizing both. He therefore divided the oxidases into two groups: (1) the guaiacum-oxidases, and (2) the amino-oxidases. An example of the latter is furnished by the oxidase of yeast. In this connection Rey-Pailhade (312) was able to find only two kinds of oxidases in plant tissues, (1) those which oxidize guaiacum and (2) those which only oxidize Röhmann-Spitzer's reagent (α-naphthol and para-phenylene diamin). The first is identical with Bertrand's laccase; the second with the indophenol-forming oxidases which occur so generally in animal tissues. In this connection Pohl (329) found certain plant extracts to give the indophenol reaction, but to be incapable of oxidizing formaldehyde.

Rosell (352) distinguishes between extra-and intra-cellular ferments, and divides the oxidases into the following groups:

(1) Aldehydases (Jacoby), such as salicylase, etc.

(2) Indophenol-oxidase (Spitzer), found in aseptic pus (Achalme).

(3) Guaiacum oxidase (Schoenbein), found in plant tissues.

(4) Hydrogen peroxide ferment (Rosell), peroxidase (Linossier), indirect oxidase (Bourquelot), and possibly includes catalase under this head.

(5) The glycolytic ferment (Lepine).

(6) The purin oxidase (Spitzer).

The following classification of the oxidases and other oxygen-carriers concerned in biological oxidations would seem to be more in line with present requirements:

(1) Laccase; ferments oxidizing guaiacum, guaiacol, hydroquinone, phenolphthalin, tannin, etc., directly by means of atmospheric or dissolved oxygen and without the intervention of hydrogen peroxide. According to Bach and Chodat, laccase consists of a peroxidase, together with an oxygenase (a peroxide). In this connection, see also a recent communication by Moore and Whitley (306).

(2) Tyrosinase; ferments acting on tyrosin and related substances.

(3) Aldehydase; ferments oxidizing aromatic aldehydes and related compounds.

(4) Indophenol oxidase (Spitzer), ferments oxidizing a mixture of α-naphthol and para-phenylene diamine to indophenol and other substances.

(5) The purin oxidases.

(6) Glycolytic ferments, causing the disappearance of sugar from animal tissues.

In addition to the oxidases proper, we have among related ferments and carriers the following:

(1) Peroxidases; oxidizing oxidase reagents only in the presence of a peroxide, such as hydrogen peroxide. These are the indirect oxidases (Bourquelot) and the β-oxidase of Grüss. According to Moore and Whitley (306) the peroxidase is the only type of enzyme concerned in oxidizing processes occurring in living cells and tissues.

(2) Catalases (Loew). These ferments actively decompose hydrogen peroxide, but are incapable of effecting the oxidation of oxidase reagents by means of the peroxide.

(3) Oxygen carriers (not true ferments). This class includes such substances as the iron-containing pigments of the blood, hemocyanin, and the γ-oxidases of Grüss. They activate the oxygen of a peroxide, but differ from the true peroxidases in having greater stability. (*See* Kastle (240).)

Whether the laccase, tyrosinase, aldehydase, etc., obtained from different vegetable and animal sources are really in all cases the same substances can not be determined with certainty in the present state of our knowledge respecting these substances. As already indicated, a very large number of plant tissues contain an oxidase capable of bluing guaiacum and oxidizing hydroquinone; whether this is the same chemical substance which is responsible for these oxidations in all cases can not be definitely decided. For certain reasons it would seem probable that the substances responsible for such oxidations, in case there were several, are at least closely related chemically. On the other hand, certain facts are known which would seem to indicate that they might have the greatest diversity of composition and yet all be capable of activating oxygen toward guaiacum, hydroquinone, etc. From what is known regarding the effect of manganese on laccase, it would seem that almost any colloidal solution of manganese might exhibit essentially the same reactions as laccase. (See pp. 122–131.)

The conduct of the oxidases and peroxidases toward a large number of substances has been investigated by Bertrand, and also by Bourquelot and others. In this connection Bertrand (52) has studied the influence of the chemical constitution of certain organic compounds on their oxidizability by laccase.

Thus, he has shown that the oxidizability of the polyatomic phenols by laccase depends upon the relative ease with which they are converted into quinones. In this respect they conform in general to the findings of the Brothers Lumière (279) respecting the action of such substances as photographic developers. Thus, para-amido-phenol is an excellent developer and is also readily oxidizable by laccase, whereas meta-amido-phenol is not a photographic developer and is not acted upon by laccase. Similar results have been obtained with para-phenylene diamin and meta-phenylene diamin. On the other hand, the aromatic monophenols and monamins are not as a rule easily oxidized by laccase; the only substances easily oxidized by laccase are those of the benzene series containing hydroxyl or amino groups in the ortho or para positions. This rule defines the oxidizing power of laccase and serves to distinguish this soluble oxidizing ferment from the oxidases which attack compounds of different chemical constitution.

Bourquelot (74) has also investigated the action of the oxidizing ferments found in *Russula delica*, namely, laccase and tyrosinase, on a large number of compounds. He found the oxidizing power of this mixture of oxidases to be dependent upon the reaction of the medium. Thus, a solution of anilin was scarcely oxidized at all, owing to its alkalinity, while in the presence of small amounts of acetic acid the oxidation proceeded rapidly up to a certain limit of acidity, beyond which it was again checked, owing to the presence of an excess of acid. He found that the mixture of oxidizing ferments from *Russula delica* is capable of oxidizing a large number of phenols (77, 79) and aromatic amino compounds (82), among which may be mentioned phenol, the cresols, xylenols, thymol and carvacrol, alpha- and beta-naphthol, also the ethers of various phenols (79), anisol, phenetol, guaiacol, acetyl-guaiacol, veretrol, eugenol, acetyl-eugenol, vanillin, and vanillic acid; and by means of aqueous extracts of *Russula delica* and *Lactarius velutinus* he was able to obtain powerful oxidations with anilin, sulfate of anilin, methyl-, ethyl-, and diethy-anilin, meta- and para-toluidin, the xylidins, naphthylamin, and veratrylamin.

According to Bertrand and Bourquelot (64, 78) tyrosin is the best reagent for tyrosinase.

OXIDASE AND PEROXIDASE REAGENTS.

Under this head are given the names of the greater number of reagents which have been actually employed in the study of oxidases, peroxidases and other oxygen catalysts occurring in the living organism. As will be seen the greater number of these substances belong to the aromatic series, and on oxidation by the oxidase or peroxidase give rise to a colored substance; thus, guaiacum is converted into guaiacum blue, phenolphthalin into phenolphthalein, which is red in alkaline solution, the leuco-base of malachite green into malachite green, guaiacol into guaiacol-tetraquinone $[C_6H_3(OCH_3)O]_4$, which is red in color. No attempt has been made to classify these reagents either as to chemical constitution or according to the nature of the substance which they yield on oxidation by the oxidase or peroxidase.

Table of oxidase and peroxidase reagents.

Name of substance.	References.
Guaiacum	51, 74, 135, 136, 332, 377, 384, 417, 418, 419.
Guaiaconic acid	142, 362
Guaiacol	75, 76
Hydroquinone	48, 49, 76.
Aloin	238, 251, 363, 364.
Phenolphthalin	240, 247, 334, 335, 336.
Ethyl-phenolphthalin	238, 245.
Leuco-rosolic acid	245.
Leuco-base of malachite green	11, 104, 105, 131, 193.
Tyrosin	64, 77, 78.
Benzidin	11, 390.
Pyrogallol	30, 31.
Potassium iodide and starch	19, 377, 379, 414.
Tetramethyl-para-phenylene diamin	464.
Para-phenylene diamin	444
Para-diethyl-para-phenylene diamin	437.
α-naphthol	77, 194.
α-naphthyl amin	390.
Vanillin	210, 266, 390, 453.
Phenol	11, 76
Pyrocatechin	11.
Ortol	43.
Amidol	258.
Tannin	272.
Salicylic aldehyde	5, 225, 291, 292, 366.
Benzyl alcohol	366.
Formic aldehyde	329.
Arsenious acid	408.
Pyramidon	252.
α-naphthol and para-phenylene diamin in solution in sodium carbonate, and analogous reagents	351, 453.
Eugenol	76, 125.
Iso-eugenol	126.

Name of substance.	References.
Thymol	77, 124.
Ortho-toluidin	74, 76.
Para-toluidin	74, 76.
Meta-toluidin	76.
Ortho-, meta-, and para-cresols	76.
Xylidin	76.
Anilin	74, 76.
Ortho-, meta-, and para-xylenols	77.
Carvacrol	77.

Many of these reagents have found special application in the study of particular phases of biological oxidations. Thus, hydroquinone and guaiacum have been most extensively used in the investigation of laccase; tyrosin in the study of tyrosinase; and salicylic aldehyde in the study of aldehydase. So, in the same way, guaiacum, aloin, benzidin, the leuco-base of malachite green, and phenolphthalin have been most extensively used in testing for blood, whereas the potassium iodide-starch reagent (v. Storch (414)), para-phenylene diamin, and guaiacum, have been most extensively employed in distinguishing between raw and boiled milk.

Various attempts have also been made to determine quantitatively the oxidizing power of these various catalysts by means of certain of these reagents. Thus, Laborde (256) has proposed a colorimetric method in which tincture of guaiacum is used for this purpose; similarly Slowtzoff (404) has employed the indophenol reaction, Kastle (239, 240, 241), the oxidation of phenolphthalin, and Czyhlarz and von Fürth (131) the oxidation of the leuco-base of malachite green, as the basis of colorimetric methods. Bach (19) has made use of the oxidation of hydriodic acid (potassium iodide and acetic acid), for quantitative purposes, and later Bach and Chodat (30, 31) have employed the change of pyrogallol to purpurogallin for the quantitative study of the peroxidase reaction. Herzog and Meier (210) have measured such oxidations quantitatively by means of vanillin, which is converted into dehydrovanillin, and Battelli and Stern (35) have employed the oxidation of formic acid. The conversion of tyrosin into melanin has also been studied quantitatively by von Fürth and Jerusalem (178), and by Bach (24). (See pp. 79–81.)

LACCASE.

This oxidase has been so fully considered in the historical development of the subject of the oxidases that but little additional need be said concerning it. As already pointed out in the foregoing, it rapidly blues guaiacum and oxidizes hydroquinone with the absorption of oxygen, and its activity is greatly augmented by the presence of very small amounts of manganese salts. Like other ferments, its activity is lost on boiling. As is evident from the work of Planche (326, 327), Schoenbein, (377, 378, 383), and Bertrand (51), laccase or a guaiacum-bluing ferment of similar nature is almost universally distributed in the plant kingdom. Thus Bertrand found it in the tubers of the dahlia, in the potato, in the rhizome of the American reed, in the racines of the beet and turnip, in the stalk of the asparagus, and in apples, pears, etc. As a matter of fact, there are certainly but comparatively few of the higher fungi or of the chlorophyllous plants which do not blue guaiacum at least in certain of their tissues at certain stages of their development.

It was believed at one time by Rey-Pailhade (342, 344) that animal tissues have not the power to blue guaiacum and that therefore they contain no laccase. Subsequent investigations, however, have shown that this is much too sweeping a generalization. Thus Biedermann (67) observed that an aqueous extract of the middle intestine of the starving meal-worm gives an intense blue coloration with guaiacum. According to Giard (192), *Botrylloides cyanescens* and *Ascidia fumigata*, Grube, give an immediate and very intense blue color with tincture of guaiacum. The blood of the last-named ascidian was clear yellow when freshly drawn, becoming dark green on exposure to the air. Portier (330) has confirmed these observations on *Ascidia mentula*, and Abelous and Biarnes (6) have obtained similar results with the blood-plasma of the craw-fish. Similarly Pieri and Portier (324) have found a powerful oxidase in the gills and labial palps and blood of the acephalous molluscs, including the common oyster and the fresh-water species, *Anadonta cygnea;* this oxidase is said to give an intense blue coloration with guaiacum and to rapidly convert hydroquinone into quinone and quinhydrone. Hougonenq and Paviot (220) claim to have found that certain malignant tumors give the guaiacum reaction; Cavazzani (113) has made experiments indicating the presence of an oxidase in the cerebro-spinal fluid, which he has termed "Cerebro-spinase," and recently the brothers Lumière and Chevrotier (281) have prepared a protoplasmic extract of red blood corpuscles to which they have given the name "Hemoplase" and which they claim possesses the properties of an oxidase to a remarkable degree, as shown by its power to oxidize guaiacum, guaiacol, paraphenylene diamin, pyrogallol, and hydroquinone. Gessard (185) has found laccase in the ink-gland of the cuttle-fish.

While these observations are of interest as showing the occurrence of laccase or a similar guaiacum-bluing ferment in animal tissues and fluids, it is undoubtedly true that such ferments are of much rarer occurrence in animal than in plant tissues. At present the precise significance of this is only a matter of conjecture. It is interesting to note in this connection, however, that Ehrlich (157) found the tissues of the higher animals to possess powerful reducing properties, as indicated by their conduct toward methylene blue. It is quite conceivable, therefore, that such oxidases as laccase are not really absent from animal tissues, but that their presence is merely obscured by the presence of powerful reducing substances, whose affinity for their oxygen is so great as to prevent the oxidation of the oxidase reagent.

Laccase is very soluble in water and aqueous solutions of the ferment are very readily obtained from such plants as the potato (*Solanum tuberosum*) (tuber), from the fruit of the egg plant (*Solanum melongena*), from the silk of the green corn (*Zea mays*), and from many varieties of fungi. In my own experience *Lactarius piperatus* and *Lepiota americana* afford excellent material from which to obtain the ferment. The former yields nearly colorless water-clear extracts of considerable oxidizing power, while the latter fungus affords extracts of remarkable oxidizing power. In this connection glycerin extracts of *Lactarius piperatus* and *Lactarius volumen*, which had been kept in the laboratory in glass stoppered bottles for a period of four years, still showed the laccase and tyrosinase reactions strongly. (See p. 56.) It not infrequently happens that laccase occurs in association with tyrosinase in the same plant or even animal tissues. See Gessard ([185, 186]), and Bertrand ([54]). In the separation of the two oxidases advantage is taken of the greater stability of laccase toward heat and alcohol. Thus from *Russula delica* Bertrand separated the two ferments by precipitating the aqueous extract obtained by macerating the fungus with its own weight of chloroform water, with one and one-half times its volume of 95 per cent alcohol. The filtrate thus obtained after concentration at 50° C., still showed all of the reactions of laccase. It failed, however, to oxidize tyrosin. On the other hand, an aqueous extract of the alcoholic precipitate oxidized tyrosin, but failed to oxidize hydroquinone or pyrogallol to any appreciable extent.

Similarly it was found possible to destroy the tyrosinase and leave the laccase in the aqueous extract by heating to 70° C. (*See* Bach [23]). In the preparation of laccase no special precautions are necessary other than those which hold for the preparation of ferments in general. If tyrosinase is present along with the laccase, the extract is either heated to 70° C. for a short time to destroy the tyrosinase, or the latter is precipitated by the cautious addition of alcohol. The tyrosinase-free extract may then be concentrated by evaporation at low temperatures and the laccase finally precipitated by the addition of alcohol. Inasmuch as we have no criterion for judging of the absolute purity of a ferment, it is very doubtful whether much is gained by the attempt to isolate laccase and the other oxidases in pure condition, and it has been my own experience that we frequently lose in activity what we gain in the so-called purity of the enzyme by all attempts at its purification. The stability of laccase apparently depends upon the nature of the substances with which it finds itself in association or upon conditions which at present are altogether unknown to us. Thus it is not an unusual thing to find that extracts of the peel of the potato lose their power to oxidize guaiacum after standing a few hours, (*see* Kastle and Shedd [247]), whereas aqueous extracts of *Lepiota americana* retain their activity for weeks or even months (*see* Kastle [238]), and glycerin extracts of *Lactarius piperatus* and *Lactarius volumen* were found by Kastle to blue guaiacum four years after they were first prepared.

Reference has already been made to the fact that aqueous extracts of laccase have been found to lose their activity on boiling. It has also been pointed out that laccase is less sensitive toward heat than tyrosinase. That it has a rather high thermal death point as compared with certain other ferments may be seen from the following observations bearing on this point. Kastle (238) found that exposure to a temperature of 80° C. or higher for a short time is sufficient to render the very active oxidases of *Lepiota americana* inert. On the other hand, at temperatures below 80° C. an hour's exposure was found to be insufficient to destroy the oxidizing power of these extracts toward guaiacum. Bertrand (cited by Green (199), p. 293) found laccase to be still active after heating to 70° C.

As already pointed out (see pp. 59–61), laccase is not specific in its action, but promotes the oxidation of a large number of easily oxidizable substances, such as guaiacum, guaiacol, phenolphthalin, hydroquinone, pyrogallol, adrenalin, and many other phenols and amino derivatives of the benzene series.

It has long been known that such substances as hydrocyanic acid and hydrogen sulfide destroy the activity of the oxidases and similar catalysts. Bouffard (69, 70) has pointed out that sulfurous acid prevents the action of oenoxydase. Up to the present, however, very little systematic work has been done on the effect of poisons on laccase. Kastle and Loevenhart (244) observed that the oxidizing power of aqueous extracts of the potato is destroyed by hydrocyanic acid, hydroxylamin, phenylhydrazin, sodium thiosulfate, and tenth-normal solutions of certain acids, such as hydrochloric, hydrobromic, benzene-sulfonic, para-nitro-toluene-sulfonic, oxalic, and salicylic, and that these substances also inhibited the oxidizing power of certain organic peroxides. Recently Bertrand (61) has found that most acids exert a poisonous or paralyzing action on laccase. On the other hand, there are some acids, such as carbonic, boric, and phosphoric, which are inactive at all concentrations.

THE PREPARATION OF LACCASE.

Slowtzoff (191), using the potato and cabbage as sources of the ferment, employed the following method for the preparation of what he calls pure laccase: One kilogram of fresh-washed potatoes were macerated to a paste and acetic acid added, so as to form a 0.5

to 1.0 per cent solution, in order to prevent the action of the oxidases on tyrosin and other chromogens contained in the potato. After standing twenty-four hours the mass is strained through a cloth and filtered. The clear reddish or yellowish liquid is then saturated with ammonium sulfate, and the precipitate, consisting of proteids, coloring matter, and ferments, is collected on a filter and washed with saturated ammonium sulfate solution, and finally dissolved in water. This salting out with ammonium sulfate and re-solution in water is repeated three or four times. The water solution finally obtained was then dialyzed against running water in pergament paper and then precipitated with 4 to 5 volumes of 95 per cent alcohol. The precipitate thus obtained was collected on a filter, washed with ether and dried over sulfuric acid. At the end of a week the yellowish-brown powder thus obtained was extracted with distilled water; a water-clear solution was thus obtained which, after saturation with chloroform and standing a month, gave only a slight percipitate (not weighable). In order now to obtain the pure ferment, the water extract thus obtained was precipitated with 5 or 6 volumes of ethyl alcohol, and the precipitate collected and dried over sulfuric acid in a desiccator. The yield of pure laccase was so small, however, that even after a year only about 1 gram of the material was obtained. The yield of laccase from cabbage was found to be even smaller.

The pure laccase obtained by Slowtzoff was found to give all of the reactions for protein and to contain 12.8 per cent nitrogen and 0.53 per cent of sulfur, and to be very poor in ash. According to this author, it belongs to the group of albumins and contains neither manganese nor phosphorus.

In order to determine the oxidizing power of his pure preparation, Slowtzoff made use of Röhmann's reagent, viz., a solution of para-phenylene diamin and meta-toluylene diamin in sodium carbonate solution, the quantity of coloring matter produced by the action of the ferment or other oxidizing agent, such as a ferric salt, being estimated colorimetrically. On the basis of these observations, he arrived at the following conclusions respecting the nature of laccase and its mode of action:

(1) Laccase belongs to the group of ferments for the reason—

- (*a*) That it loses its activity at high temperatures. In this connection he found the thermal death point of the ferment to vary with the degree of purity of the preparation; thus his purest preparations lost their activity at as low a temperature as 50° C., whereas preparations richer in ash only lost their activity at temperatures of from 65° to 70° C.
- (*b*) The amount of the substance oxidized is proportional to the square root of the quantity of laccase present.
- (*c*) The quantity of product resulting from the action of the ferment is proportional to the quantity of the ferment, but not to the amount of substances being oxidized.

(2) The pure laccase preparations were found to act best in weakly alkaline solutions, as already observed by Bertrand and Bourquelot.

(3) Laccase belongs to the group of proteins. Its ash constituent is very small and without influence on its oxidizing power.

(4) Laccase is not destroyed by weak acids, nor by peptic or pancreatic digestion.

ANTI-LACCASE.

According to Gessard ([184]) it is possible to obtain a serum capable of retarding the action of laccase by the injection of preparations containing this oxidase subcutaneously into a rabbit. In order to obtain this anti-laccase serum Gessard adopted the following mode of procedure: A rabbit weighing about two kilograms received at intervals of five or six hours apart six injections, each of 1 gram of laccase powder in 10 c. c. of water. According to Bertrand ([55]) this represents about 0.15 gram of pure laccase. Two animals treated in this manner gave a serum of about the same potency. By means of guaiacum and guaiacol he found that the serum obtained from animals thus treated completely retards the action of laccase when two parts of the serum by volume are added to one part by volume of a 2 per cent solution of laccase. On the other hand normal serum and anti-tyrosinase serum were both found to be without effect on the action of laccase. Anti-laccase prepared by the use of the laccase from the juice of the lac tree was found to retard to a degree at least the color reactions produced by an extract of *Russula delica* on oxidase reagents. On the other hand Gessard ([185, 186]) observed that the anti-laccase obtained by the injection into rabbits of laccase from the lac tree is without retarding action on the laccase from the ink gland of the cuttle fish. He concludes therefore that the oxidases do not differ from other enzymes in their power to give rise to specific anti-bodies in the blood serum of animals, which have received a number of injections of the ferment.

According to Gessard the results of these researches on anti-laccase and anti-tyrosinase are sufficient to prove the individuality of these two oxidases, and serve to show that the oxidases do not differ from other enzymes in regard to their power of giving rise in the serum of animals to substances which oppose their action.

Czapek ([129, 130]) has also obtained evidence of the production of anti-oxidases in plants which hinder the oxidation of homogentisic acid, and which are produced in the growing ends of roots, special sense organs, and in fungi, as the result of irritation.

In the light of Bach and Chodat's views regarding the nature of the oxidases, the production of anti-bodies in the serum of animals as the result of the repeated injection of oxidases is probably due to the action of the peroxidase moiety of the oxidase and not to the oxygenase, since of these the former only seems to possess the properties of a ferment. Gessard's work is of such importance as to warrant confirmation, especially in view of the failure of von Fürth and Jerusalem (178) to obtain evidence of anti-tyrosinase, and the whole subject of anti-oxidases should be reinvestigated by means of Bach's peroxidase.

TYROSINASE.

It has long been known that on exposure to the air certain of the higher fungi turn pink or red and finally black, whereas other species become blue. We have already seen that the latter change is due to an oxidizing ferment, laccase. That the reddening and final blackening of other species of mushrooms is due to the action of a specific oxidase was first suspected by Bourquelot and Bertrand (89), who in 1896 pointed out the existence in certain mushrooms, such as the *Rassula foetens* Pers., of a very active oxidizing ferment, probably different from laccase. They (87, 88) then showed that the blackening of *Russula nigricans* differs from the bluing of *Boletus cyanescens* by reason of the fact that the blackening of the crystalline chromogen contained in the former species is not accomplished by the laccase of the sap of the lac tree, whereas the blackening is undoubtedly due to an oxidation, as indicated by the fact that if one does not agitate the aqueous extract of the fungus the blackening takes place first in the upper layers of the liquid, and during the blackening oxygen is absorbed. Continuing these investigations Bertrand (53) proved the crystalline chromogen of *Russula nigricans* to be tyrosin. He also found that the beet root and the tubers of the dahlia and potato, like certain of the higher fungi, also redden and then turn black on exposure to the air. This change he now definitely proved to be an oxidation of tyrosin by atmospheric oxygen under the influence of a specific oxidizing ferment, to which he gave the name *tyrosinase*. From the roots and tubers of certain plants, such as the beet and dahlia, he was able to obtain in crystalline condition as much as 0.5 gram of tyrosin from one quart of the expressed juice, an amount about corresponding to the solubility of the compound in pure water. The tyrosin thus obtained was identified by Hoffmann's or Piria's reactions, and its composition determined by analysis. He also isolated tyrosin from *Russula nigricans*, and in this connection Bourquelot and Harlay (90) give a drawing of a transverse section through the stipe of *Russula nigricans*, showing the rosettes of tyrosin crystals distributed more or less regularly throughout the tissue of the fungus. Bertrand also showed that the blackening of tyrosin is due to an oxidase and that this oxidase differs from laccase. Thus when a small amount of the aqueous extract of the russula, prepared in the cold, and a solution of tyrosin were brought together, the mixture became red, then inky black, and finally deposited a black precipitate. He showed that oxygen was absorbed at the same time; this was proved by simply allowing the tube in which the reaction was being carried out to remain quiet, whereby these color changes were first observed to occur on the upper surface of the liquid. In a vacuum or under a bell jar, resting in a watch glass on the surface of mercury, the mixture acquired a faint rose tint, since it was impossible to remove all traces of oxygen therefrom, but they showed no further deepening of color no matter what the duration of the experiment. With a boiled extract of russula no change of color was observed. A repetition of these experiments on the boiled juice of the beet, or with tyrosin of animal origin (from the horse), or of vegetable origin (from the dahlia or russula), or with the oxidase obtained from the beet or the dahlia, always led to the same results. Finally, in order to prove that the oxidation of tyrosin could not be accomplished by laccase, the following experiment was carried out: A certain amount of extract of russula was introduced into a vacuous flask, and then some tyrosin added. The flask containing these substances was then allowed to stand for twenty-four hours, at the end of which time no change of color had occurred. The contents of the flask were then heated to 100° C. for ten minutes in order to destroy all enzyme action. The flask was then opened and its contents exposed to the action of the air, but the tyrosin remained unaltered even after the addition of ordinary laccase. Hence the blackening of tyrosin is not due to the successive action of two ferments but solely to that of tyrosinase in the presence of oxygen. He then points out that independently of their special interest, these observations go to show that laccase is not the only oxidizing ferment existing in the vegetable world but that on the contrary, it should be regarded as a type of a series of analogous substances to which he had already given the generic name of *oxydases*. (See p. 54.)

While perhaps not so widely distributed in nature as laccase, tyrosinase has been found in a large number of plants and animal species. Bourquelot and Bertrand (87, 88, 89) found it in a large number of fungi, and also in phenogams. According to Lehmann (260) and Lehmann and Sano (261) tyrosinase is found in a number of species of bacteria, notably in *B. fluorescens nonliquefaciens*, and also in *B. phosphorescens*, *B. putridens*, and *Actinomyces chromogens*. As a general thing, wherever we find tyrosinase in plant tissues, we are apt to find laccase. The converse of this, however, does not hold generally that is, we do not find tyrosinase wherever we find laccase. For example, in *Russula delica*, *Lactarius piperatus*, and in the tubers of the dahlia and potato, we find both tyrosinase and laccase, whereas in the silk of the green corn (*Zea mays*) we find laccase but no tyrosinase.

Tyrosinase is also widely distributed in the animal kingdom, where it plays an essential rôle in the formation of animal pigment (*melanogenesis*). The following are the more important investigations bearing on this point: The blackening (melanose) of the blood (hemolymph) of certain insects was made the subject of an investigation by Krukenberg ([254]) and also by Fredericq ([176]) as early as 1881. According to Fredericq, oxygen was responsible for this change of color, whereas, according to Krukenberg, it was probably due to carbon dioxide. Fredericq also made the interesting observation that the blood obtained from insects which previous to bleeding had been heated to 50–55° C. for fifteen minutes, showed no blackening on exposure to the air. Dewitz ([138]) has also shown that an oxidase, the precise nature of which was not investigated, plays an essential rôle in the metamorphosis of certain insects. As is well known, the larvæ of the fly are white during the entire life of this phase of the insect, and only at the moment of the formation of the pupa does any change of color take place. This color change begins in the abdominal cavity as two large colored spots.

Biedermann ([67]) seems to have been the first to obtain evidence of tyrosinase of animal origin. In his experiments the middle intestine (*Mitteldärme*) of three or four starving meal worms (*Tenebria molitor*) were placed in chloroform water. The light yellowish solution thus obtained was divided into two portions. To one portion a few drops of a solution of tyrosin was added, and both were kept over night in open watch glasses in a moist chamber. The portion containing the tyrosin had become violet black in color, whereas the portion to which no tyrosin had been added was only slightly darkened.

Von Fürth and Schneider ([179]) have also made important contributions to our knowledge of animal tyrosinase and its relation to animal pigmentation. The pupae of the butterfly, chiefly of the species *Deiciphila elpenor* and *euphorbiae*, furnished the material for their investigations. By careful puncture and squeezing, about one cubic centimeter of a clear, bright greenish-colored fluid was obtained from each pupa. This was the hemolymph, called for the sake of brevity the *blood* of the insect. On standing a few moments in the air this fluid began to darken on the upper surface, and after a time a black clot separated. On the other hand, if the proteid of the fresh blood be removed by boiling, it shows none of these color changes on standing in the air. When dried in vacuo over sulfuric acid, no change of color in the blood was observed. When a few drops of the fresh blood was added to a few cubic centimeters of a fresh solution of tyrosin, there appeared on the upper surface of the solution a violet-colored ring; this color gradually spreads through the solution until the whole of it is colored a dark violet, and is turbid through the separation of a fine and coarse flocculent precipitate. On the other hand, two drops of the fresh blood added to water gave a solution which showed none of the color changes, but ultimately gave a precipitate scarcely darker in color than that of the fresh blood. The diluted blood gave with tincture of guaiacum a dirty, dark bluish-green color after long standing, and also a positive reaction with Spitzer's para-phenylenediamin-α-naphthol reagent. It is therefore evident from these observations that the blood of these lepidoptera contains oxidases having the power of oxidizing guaiacum, Spitzer's reagent, tyrosin, and the chromogen of the blood itself. Attempts to purify the tyrosinase of the blood of lepidoptera by precipitation with uranyl acetate according to Jacoby (225) failed. Von Fürth and Schneider were able to separate it from the chromogen and from the crystalline substances of the blood by fractional precipitation with ammonium sulfate. The ferment thus purified gave with tyrosin a violet-colored solution, which after a short time became dark violet and finally black. It was also found to produce coloring matter with pyrocatechin (yellowish red) and hydroquinone (red solution becoming turbid and finally showing a considerable brown precipitate); a carmine-red solution of an iron compound of suprarenin, the blood-pressure-raising compound of the suprarenals, became dirty brown in color, whereas the control experiment showed no alteration. Oxyphenyl-ethyl amin (Emerson) became yellowish brown and gave finally an olive-colored precipitate. On the other hand, it was found to have no action upon casein, indicating that the ferment is powerless to act on the tyrosin residues contained in proteid molecule. The nature of the black substance produced by the action of the tyrosinase of lepidoptera on tyrosin was also studied. Ten to twenty c. c. of a freshly prepared solution of the ferment, obtained from the lymph of from twenty to forty Deiciphila pupae, was brought together with a few decigrams of finely pulverized tyrosin and shaken vigorously in a shaking machine for four to six hours. In a short time the characteristic violet coloration appeared in the solution, and after a time a considerable black precipitate separated, leaving the supernatant fluid clear and colorless. With fresh quantities of tyrosin this gave no further coloration. This material was then filtered off and washed with water, then with dilute hydrochloric acid, then with hot water until chlorine free, and finally with alcohol and ether and dried to constant weight at 110° C. This black substance was found to be insoluble in water, alcohol, ether, and the common organic solvents and in dilute alkalis at room temperatures and in strong boiling hydrochloric acid. A small quantity of it melted with the purest sodium hydroxide from sodium gave rise to an unmistakable odor of indol and skatol. On analysis it gave the following numbers:

	Per cent.
C	55.44
H	4.45
N	13.74

corresponding to the following atomic ratios:

$$N:H:C=1:4.55:4.77,$$

which is essentially the same as that obtained by Hofmeister for a whole series of pigments ordinarily called *Melanine*, to which belong the dark pigment of the hair, and of the skin, the choroid coat, melanotic tumors, sepia black, and certain split products of proteids, such as Schmiedeberg's melanine acid (melanin saüre). In its physical properties, solubility, etc., and in its tendency to yield substances having a skatol-like odor, on melting with alkali, it agrees closely with the melanins, and with a pigment obtained by Ducceschi (149) by oxidizing tyrosin with potassium chlorate in hydrochloric acid solution. For other analyses of the black pigment of hair and feathers, see Hodgkinson and Sorby (213).

According to these authors (von Fürth and Schneider) tyrosinase in its occurrence in the animal kingdom is by no means limited to the insects. They have also obtained it from the blood of the crawfish (*Flusskrebse*), and by refined methods it would doubtless be possible to recognize tyrosinase in the most widely differing classes of animals. Acting upon their suggestion, Przibram, of the zoological station at Trieste, found tyrosinase in the ink sac of the cuttlefish, *Sepia officinalis.* An extract of the washed epithelial lining of the sac gave with a solution of tyrosin first a very beautiful orange-yellow color, changing to sepia brown, and finally yielding a black precipitate. Von Fürth and Schneider are therefore of the opinion that probably wherever melanotic pigments occur in the living tissues of the lower and higher animals they originate as the result of the action of appropriate enzymes on substances of aromatic nature. They point out in this connection that Salkowski and Jacoby have shown independently that tyrosin results from the autolysis of various animal tissues. It would seem likely therefore that in the formation of melanotic pigments two ferments are jointly concerned, one, an autolytic ferment capable of splitting off tyrosin or a similar aromatic complex from the protein molecule, and the other tyrosinase, which transforms the tyrosin into melanin. To determine whether tyrosinase occurs in melanotic tumors offers an interesting and important field for further investigation.

Gessard (184) has obtained tyrosinase from the glands of the ink sac of the cuttlefish (*Seiches*) and calamary (*Calmar*), and in 1904 this same author (190) showed that the coloration of the integument of the green fly (*Lucilia Caesar* L.) is due to the action of tyrosinase. The larvæ of the fly are white, and from them this author was able to obtain tyrosin in crystalline condition, as well as to demonstrate the presence of tyrosinase. In the course of their metamorphoses these larvae exhibit a succession of colors similar to those shown by a solution of tyrosin when acted on by tyrosinase, until finally the insect attains the full iridescent green color of the fly. When the white pupae are kept in a vacuum the ferment is inactive and they remain white. These facts serve to corroborate the hypothesis that tyrosinase is responsible for the production of cutaneous pigments in man and animals.

In order to demonstrate the presence of tyrosinase, the ink sac (glande du noir) of the cuttle fish was removed and macerated with chloroform water and filtered through a Chamberland filter. Under these conditions the fine granules of pigment are retained by the filter and a clear solution of the ferment is obtained which exhibits the same color changes with tyrosin as is shown by an extract of russula. Gessard was also able to demonstrate the presence of tyrosinase in the commercial product known as *sepia en vessie*—crude sepia. This is simply the dried gland with its contents and is employed in the preparation of the refined coloring matter of the same name, sepia. In the course of this investigation he also showed that the anti-tyrosinase serum (182) obtained by the repeated injection of a rabbit with vegetable tyrosinase is powerless to hinder the action of the animal tyrosinase on tyrosin, indicating that, despite the similarity of the two varieties of tyrosinase in their action on tyrosin, they are not precisely alike in all respects. (See also Gessard, 183.)

Similarly in his studies on the formation of the melanotic pigments in tumors of the horse, this author (184) calls attention to the fact that a general chemical and physical relationship has long been recognized as existing between the black pigments of the eye and skin and that of the cuttle fish (Seich) and other molluscs. It is quite likely, therefore, that our knowledge of the formation of this pigment in the case of the cephalopods will hold equally well for its production in other animals. The abnormal production of melanotic pigments in healthy or diseased tissues of man is rare, but more common in those of the horse, in which case its production is of less formidable significance. Melanotic tumors are especially common on the white horse, and these furnished the material for Gessard's investigations. He has found that in the production of the melanotic pigment of such tumors the same agencies are at work as in its production in the ink sac of the cuttle fish, viz, a chromogen and an oxidizing ferment. From such tumors he was able to obtain tyrosin by appropriate methods, in crystalline condition, and aqueous extracts thereof were found to give with tyrosin the color changes characteristic of tyrosinase. The author concludes therefore that tyrosin is the chromogen whose oxidation by tyrosinase gives rise to the pigment in melanotic tumors and wherever else such pigments are met with in the animal economy. He is also of the opinion that the color of the negro is due to the reaction which gives rise to production of the ink of the cuttle fish (Seiche) and the black pigment of mushrooms. While such is doubtless the case, we are still a long way from an understanding of the physiological cause which gives rise to tyrosinase in the epidermal tissues of the negro and the lack of its general production in the epidermal tissues of the white races under normal conditions. The fact, however, that melanotic pigments are formed over limited areas in the dermal structures of white-skinned races indicates that the same causes of pigmentation may be operative in all races to a greater or less degree and serves to emphasize the importance of further studies on the mode of action of tyrosinase and its mode of origin in animal tissues.

The occurrence of tyrosinase in the skins of certain pigmented vertebrates has been investigated by Miss Florence M. Durham (155). Aqueous extracts of the skins of rabbits, rats, guinea pigs, and chickens in the fœtal stage of development, containing small amounts of ferrous sulfate, were found to act upon tyrosin with the production of pigments similar in tint to those characteristic of the coat of the animal. Thus black pigments were formed with extracts of the skins of black-pigmented animals, and yellowish pigments with those of the skins of animals containing orange-colored pigments, and with extracts of the skins of white or albino animals no pigments were formed by the action of an extract of the skin on tyrosin. The tyrosinase present in the skins of animals was found to act most rapidly at 37° C. It is destroyed by boiling and does not act in the cold. From one to ten days were required for the production of the pigment in vitro. Another peculiarity of the tyrosinase contained in the skins of animals is that it only acts upon tyrosin in the presence of small amounts of iron.

Phisalix (321) has obtained tyrosinase from the skin of the green frog, and Gessard (188) has found it in the skins of frogs of other species, such as the frog rousse and the common toad. More recently Phisalix (322) has investigated the color changes occurring in the larvæ of the cockroach (*Phyllodromia germanica*). Immediately after hatching from the egg the integument of the larva is soft and bright white in color. In the course of a short time, however, it becomes grey, then brown, and finally black, so that three hours after hatching the little cockroach is entirely black. According to this author this change of color is the result of the action of tyrosinase on tyrosin, both of which occur in the embryo at the moment of its development; indeed, it is probable that they are both present in the egg in which they are deposited at the time of ovagenesis.

Still more recently a very valuable contribution to our knowledge of melanotic pigments and fermentative melanine formation has been made by Von Fürth and Jerusalem (178). These authors have compared hippomelanin (the black pigment produced in tumors of the horse) with other natural and artificial melanins, both as to mode of formation and general physical and chemical properties. They found hippomelanin to be iron free and are inclined to look upon its sulfur content as of accessory nature. In its physical properties, general chemical conduct, and decomposition products, hippomelanin shows a close resemblance to the artificial melanin produced by the action of tyrosinase on tyrosin, whereas it differs in certain respects from other melanins and epidermal pigments and the pigment phymatorhusin of malignant melanotic tumors. Up to the present, according to these authors, the chemical investigation of hippomelanin has disclosed no fact at variance with the well-established hypothesis of the fermentative origin of melanin as the result of the action of tyrosinase on the cyclic complexes resulting from the degradation of the protein molecule.

The principal sources of vegetable tyrosinase are certain of the higher fungi, especially many species of russula, such as the *Russula delica* (Bourquelot and Bertrand, (87, 88, 89)), *Agaricus melleus* and *Agaricus campestris* (Von Fürth and Jerusalem, [178]), and wheat bran (Bertrand and Mutermilch, [65]). The ink sac of the cuttle fish (*Sepia officinalis*) and the hemolymph of the pupæ of *Deiciphila euphorbiæ* (178, p. 161) are the chief sources of animal tyrosinase. In order to obtain a solution of tyrosinase from *Russula delica* it is only necessary to macerate the fresh fungus with chloroform water or with glycerin, or, as recommended by Bach (24), the tyrosinase of *Russula delica* may be precipitated from the aqueous extract of the fungus by means of 96 per cent alcohol. Young, sound fungi are ground in a sausage machine and 300 c. c. of the clear expressed juice is poured into 1.5 liters of 96 per cent alcohol. The precipitate thus formed is filtered off by means of a filter pump, washed with alcohol, and dried in vacuo over calcium chloride. The dried precipitate is now mixed with 300 c. c. of water, whereby only a small amount of the material goes into solution. The mixture is then filtered, an entirely colorless solution of tyrosinase being obtained.

In this connection Bach (24) has observed that the activity of the tyrosinase solutions depends on the age and state of preservation of the fungus. Thus from three lots of the fungus (I, young, unblemished fungi; II, older, more or less injured fungi; and III, putrid fungi) he obtained aqueous extracts, equal amounts of which acting on the same quantities of tyrosin, required the following quantities of 0.002 N. permanganate:

I.	II.	III.
37.8 c. c.	13.6 c. c.	8.3 c. c.

The residue was then dried in vacuo or at once dissolved in 100 c. c. of chloroform water, in which case it was used at once, since aqueous solutions of the ferment are very unstable. By this mode of preparation the tyrosinase is separated from the catalase which the fresh aqueous extracts of this fungus contain. These authors have found that tyrosinase is easily destroyed or rendered inactive by alcohol, and hence if the ferment is prepared by this method it is necessary to filter off the alcohol as soon as practicable.

In order to prepare tyrosinase from the ink sac of the cuttle fish, Gessard (184) macerated the ink sac with chloroform water and filtered through a Chamberland filter. A clear solution was thus obtained which gives with tyrosin the same color changes as those shown with an aqueous extract of *Russula delica.* In the preparation of the animal tyrosinase employed in their latest investigations Von Fürth and Jerusalem (178) employed the hemolymph of the pupae of *Deiciphila euphorbiae.* This was half-saturated with ammonium sulfate, and the washed and pressed precipitate thus obtained dissolved in 0.04 per cent soda solution. This solution exhibited strong tyrosinase reactions.

In order to obtain the ferment from wheat bran, Bertrand and Mutermilch (65) recommend the following method: One part of wheat bran is mixed with four parts of water and the mixture allowed to stand for several hours. The mixture is then centrifugalized, and the solution thus obtained is mixed with three volumes of 95 per cent alcohol and again centrifugalized. The precipitate is then washed with 80 per cent alcohol, mixed with distilled water, and again centrifugalized. The solution thus obtained is then mixed with three to four times its volume of alcohol, and the precipitate thus formed is collected, washed with strong alcohol, and dried in vacuo over sulfuric acid. The substance thus obtained, the yield of which is about 0.8 per cent, contains no laccase. On the other hand, when dissolved in water and filtered through a Chamberland filter, a clear solution is obtained which of itself undergoes no alteration on exposure to the air. On the addition of small amounts of tyrosin, however, it passes rapidly through a succession of colors—rose, cherry red, and finally, brownish black. On the other hand, if all gaseous and dissolved oxygen be removed, or if the filtered solution be heated to 100° C. for five minutes, no coloration with tyrosin is observed.

In the preparation of vegetable tyrosinase from *Agaricus melleus,* Von Fürth and Jerusalem (178) rubbed up two kilograms of the fungus with sand, and extracted the mass with two liters of chloroform water. After two or three hours the supernatant liquid was poured off and mixed with twice its volume of 96 per cent alcohol. The precipitate was filtered off on raw silk.

All observers seem to be agreed that tyrosinase is a true enzyme. Thus it appears to conform to Portier's (330) definition of an oxidizing ferment, according to which, first, gaseous or dissolved oxygen is necessary for its action; second, its action is accompanied by a notable absorption of oxygen; third, it is destroyed by heat; and, fourth, it is nondialyzable.

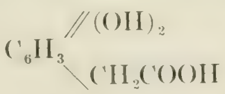
Miss Durham (155) found that after filtering off the pigment produced by their action upon tyrosin, the preparations of animal tyrosinase could act upon fresh portions of tyrosin. According to Bertrand (59) tyrosinase is more easily destroyed by heat than laccase; thus he found the former to be destroyed in twelve minutes at 60–70° C., whereas laccase withstood this temperature for twenty hours. Gessard (182) found tyrosinase to be almost destroyed by heating to 65° C. for thirty seconds. Chodat and Staub (117) observed the activity of tyrosinase to increase with a rise of temperature to 61° C., and to become inactive at 61° C. Advantage has been taken of this difference in the stability of tyrosinase and laccase toward heat in the separation of the two enzymes, and tends to show that these two oxidases are different ferments. Thus *Russula delica* contains both tyrosinase and laccase. If now, according to Bertrand (54) the fungus be macerated with its own weight of chloroform water and the liquid thus obtained be treated with alcohol in the proportion of three volumes of alcohol to two volumes of extract, a precipitate is obtained which gives the reactions of tyrosinase. If now the filtrate be evaporated at 50° C. to one-tenth of its original volume, it will be found to act energetically on hydroquinone and pyrogallol, but to be without action on tyrosin. In other words, the filtrate that has been subjected to a temperature of 50° C. for some time still contains laccase, but no tyrosinase. Similarly Bach (25) has obtained laccase free from tyrosinase from the fungus *Lactarius vellereus* by heating to 75° C. Miss Willcock (451) has shown that tyrosinase is not killed by the radium emanation.

Kastle has observed that glycerin extracts of *Lactarius piperatus* and *Lactarius volumen* oxidize tyrosin strongly after having been kept four years in glass-stoppered bottles under ordinary laboratory conditions.

Like many other enzymes, tyrosinase is sensitive to the action of acids, alkalies, salts, and certain poisons like hydrocyanic acid. Thus Wolff (459) finds that the tyrosinase from *Russula delica* is most active on tyrosin when the solutions are neutral to phenolphthalein. Similarly Abderhalden and Guggenheim (1) find that N/100 hydrochloric acid inhibits the action of tyrosinase, and that N/100 sodium hydroxide retards it considerably. After treatment with acid or alkali, neutralization of the acid or alkali fails to restore it to its original activity, indicating that the ferment is actually destroyed under these conditions. Gessard (182) found that while salts of the metals promote the coagulation of the black pigment, they hinder the development of the initial coloration, the retardation being proportional to the quantity of salt present. Neutral salts were found to produce a retardation ranging from twenty-three minutes to nine days, and alkaline carbonates prevented the development of the color for seventeen days. Even egg albumin and blood serum were found to retard the action of the ferment.

The changes of color produced by the action of tyrosinase on tyrosin have been carefully studied by Gessard (183). According to this author, when tyrosinase is added to an aqueous solution of tyrosin, oxygen is absorbed and the liquid takes on a rose color which gradually becomes reddish yellow, rapidly changing to mahogany red and then to garnet. He also observed the most marked coloration in the upper layers of the liquid, due to immediate contact with the air. Continuing these studies, Gessard (189) observed the coloration of tyrosin by tyrosinase to consist of two distinct phases, the first of which alone, viz, the change to rose and then to red, is, according to this author, attributable to the ferment. After a certain interval, depending on general conditions, the solutions acquire a violet color, and finally yield a black precipitate (melanine), leaving the supernatant fluid perfectly colorless. When, for example, the red liquid resulting from the action of tyrosinase upon tyrosin is exposed to a vacuum, it slowly becomes colorless. Now, on exposure to the air, the decolorized solution acquires a violet instead of a red color. Hence, a new substance has been produced by the reduction of the first substance in the vacuum, from which the first substance apparently can not be regenerated. The substance thus obtained by exposing the reddened solutions of tyrosin to a vacuum has been found to be very oxidizable and is characterized by its yielding a violet solution on oxidation, the coloring matter showing a great tendency to separate from the solution in the form of a black precipitate. The production of the violet compound as the result of the action of tyrosinase on tyrosin is also facilitated by the presence of certain salts, a fact which would probably explain the production of the black compound under natural conditions.

Aside from the fact that melanines are formed by the oxidation of tyrosin through the agency of tyrosinase, but little is known as to the precise mechanism of the process. According to Gonnermann (195), homogentisic acid, hydroquinone-acetic acid—



$$C_6H_3\begin{matrix} /\!\!/(OH)_2 \\ \diagdown CH_2COOH \end{matrix}$$

is the principal product resulting from the action of tyrosinase on tyrosin in the presence of oxygen. According to this author, tyrosinase is not an oxidizing but a hydrolytic ferment, and his conception of the process is that the homogentisic acid produced by the action of tyrosinase on tyrosin in the presence of air results from the spontaneous oxidation of an unknown product of the hydrolysis of tyrosin by tyrosinase and not from the direct oxidation of the tyrosin, through the agency of the ferment. This view of the mode of action of tyrosinase has recently been disproved by Bach (25), who has shown that tyrosinase does not produce black pigments from mixtures containing such substances as might be produced by the hydrolysis of tyrosin, such as phenol + d + l serin, hydroquinone + alanin, p-cresol + oxyamino-acetic acid, and p-oxy-benzyl alcohol + glycocoll.

Gonnermann's hypothesis respecting the mode of action of tyrosinase has also been refuted by Chodat and Staub (117). The results of their experiments in an atmosphere of carbon dioxide show clearly that oxygen is required for the process and that the action of the ferment is not simply the production of an easily oxidizable substance by the hydrolysis of tyrosin.

We have seen that according to Gessard (181, 189), the action of tyrosinase on tyrosin consists of two distinct processes, first, the oxidation of tyrosin to a red substance, and, second, the condensation of the red substance to a black product (melanine). The oxidation can be accomplished either by tyrosinase in the presence of air, or by a chemical oxidizing agent such as Millon's reagent, whereas, for the condensation of the red substance into the black pigment, the action of the mineral salts contained in the tyrosinase are necessary. This hypothesis respecting the mode of action of tyrosinase has recently been refuted by Bach (25). In order to throw further light on this subject, he prepared an active solution of tyrosinase and heated it to boiling. After cooling, tyrosin was added to the solution, and 20 c. c. portions of it were placed in four reagent glasses. To the first of these there was then added 5 c. c. of water, to the second 2 c. c. of 1 per cent hydrogen peroxide and 3 c. c. of water, to the third 2 c. c. of 1 per cent hydrogen peroxide solution, 2 c. c. of a peroxidase solution, and 1 c. c. of water, and to the fourth a solution of an oxidase prepared by heating an extract of *Lactarius vellereus* to 75° C. to destroy the tyrosinase. These tests remained colorless for weeks, indicating that the oxidation of tyrosin by tyrosinase is not referable to the inorganic substances which it contains. Bach also sought in vain for a co-ferment of tyrosinase among plants. He concludes, therefore, that the oxidation of tyrosin only takes place through the action of peroxidases, hydrogen peroxide, plant juices, or ferment preparations when the preparation itself is active to tyrosin, that is, when it contains tyrosinase. Indeed, it would seem from his most recent utterances on the subject that Bach (ibid.) is inclined to look upon the action of tyrosinase as completely different from that of the common oxidases (peroxidase + hydrogen peroxide). Tyrosinase, he says, belongs to a peculiar class of oxidizing ferments, whose oxidizing action is exerted upon substances containing slightly labile hydrogen.

Formerly all observations upon the action of tyrosinase were qualitative in character, being confined to rather crude and indefinite color comparisons. Recently, however, three quantitative methods for determining the quantity of pigment resulting from the action of tyrosinase on tyrosin have been proposed. These are (1) a spectrophotometric method, (2) a sedimentation method, and (3) a volumetric method by means of a 0.002N solution of potassium permanganate. The first two methods were proposed by Von Fürth and Jerusalem (178). For details concerning these methods and the use of the instrument, with which the writer is not familiar, the original article may be consulted. In this connection see also Mörner (307). The second method of Von Fürth and Jerusalem (178) depends upon the sedimentation of the pigment by boiling with a small amount of calcium chloride. After boiling a short time the beakers or tubes containing the substances are allowed to stand, when the black pigment settles out, leaving the liquid clear. The clear supernatant liquid is then poured off and the residue washed with water and transferred to a graduated centrifuge tube by means of water, centrifuged and measured. The third method was first employed by Bach (23), and depends upon the fact that the brown pigment formed by the action of tyrosinase on tyrosin can be oxidized by a dilute acid solution of permanganate, 0.002N, to a colorless compound. Hence in order to determine the quantity of pigment produced in a given time by the action of tyrosinase on tyrosin, the black mixture is titrated with 0.002N permanganate, after the addition of sulfuric acid, until the color of the titer disappears.

Of the three methods, Bach's is the simplest and commends itself most strongly to chemists. It requires no special apparatus, and by its use he (Bach) obtained far more regular and generally concordant results than Von Fürth and Jerusalem were able to obtain with the spectrophotometric and sedimentation methods. (See Bach 24).

THE KINETICS OF MELANINE FORMATION BY TYROSINASE.

The quantitative methods devised by Von Fürth and Jerusalem (178) and by Bach (23) have already been utilized by these observers and also by Chodat and Staub (117) in studying the kinetics of the tyrosinase process. According to the latter the velocity of the reaction at small concentrations is proportional to the quantity of ferment present. At greater concentrations the rate of the reaction has been found equal to the algebraic expression, $Kc + b$, in which c is the concentration, and K and b are constants. Von Fürth and Jerusalem (supra) have investigated the effect of temperature, quantity of ferment, and the influence of hydrogen peroxide, alkalinity, and inorganic catalyzers on the kinetics of melanine formation by vegetable and animal tyrosinase.

Von Fürth and Schneider (179) found the tyrosinase of insect blood to be exceedingly sensitive to the prolonged action of high temperaturetures. Thus a long exposure to 30° C. is sufficient to inhibit the action of the ferment. In order to determine the effect of temperature on the action of vegetable tyrosinase (from *Agaricus melleus*) Von Fürth and Jerusalem prepared four tubes, each of which contained 4 c. c. of the tyrosinase solution, 60 c. c. of an alkaline solution of tyrosin, and 2 c. c. of 3 per cent hydrogen peroxide. These tubes were labeled *a*, *b*, *c*, and *d*; *a* was kept at 5°–7° C., *b* at room temperature, *c* at 40° C., and *d* at 55° C. At the end of half an hour *a* and *b* were not noticeably altered in appearance; *c* was darker, and *d* was the darkest in color. The next morning *a* and *b* were colored black, *c* was lighter, and *d* was the lightest in color. The quantities of melanine in the four tubes as determined by the spectrophotometric method were found to stand in the following ratio:

$$a = 1.29;\ b = 0.55;\ c = 0.42;\ \text{and}\ d = 0.22.$$

These authors conclude, therefore, that so far as the influence of temperature on the production of melanine by the action of tyrosinase is concerned, two opposing processes are at work, viz, the accelerating effect of temperature common to all chemical reactions and the destructive action of temperature on the labile ferment. The result is that the process reaches an equilibrium between 30° to 50° C., and that between 60° and 65° C. the ferment ceases to act on the tyrosin. The effect of small amounts of hydrogen peroxide is to increase the quantity of melanine produced by tyrosinase in a given time, whereas larger quantities of the peroxide exert a retarding effect on the process. Roughly, the quantity of melanine formed by the action of a given amount of the ferment acting under the same conditions was found to be proportional to the concentration of the tyrosin. Within narrow limits of concentration (0 to 10 c. c. of 0.04 per cent sodium carbonate solution in a total dilution of 27 c. c.) the effect of alkali was found to be practically negligible. Within certain limits the quantity of melanine formed increases with increase in the concentration of the fungus tyrosinase. Thus the effect of doubling the quantity of ferment was to cause an increase in the production of melanine from 1.0 to 1.4. On the other hand, the addition of further amounts of the ferment caused no increase in the production of melanine; in fact, a slight diminution in the quantity of melanine occurred, indicating that increasing quantities of the ferment above certain limits cause a retardation of the process.

With animal tyrosinase (from the hemolymph of *Deiciphila euphorbiae*) the rate of melanine formation was considerably increased by an increase in the quantity of hydrogen peroxide present, but in the end very nearly the same amounts of melanine were produced in all cases. Acids, even the weakest, were found to prevent the action of animal tyrosinase, while the effect of the addition of small amounts of alkali is to cause a distinct increase in the activity of the ferment. Of the metallic catalyzers tested, viz, 1 per cent solutions of the sulphates of manganese, iron (ferrous), copper, and nickel, only manganese was found to increase the rate of production of the melanine by animal tyrosinase. With the animal tyrosinase much greater increases in the quantities of melanine produced resulted from increases in the quantities of ferment; in other words, while the further addition of animal tyrosinase over and above a certain amount caused no corresponding increase in the quantity of melanine produced, no distinct hindrance of the process, such as that brought about by large amounts of vegetable tyrosinase, was observed.

As already indicated in the above, Bach's (23) permanganate method for the determination of melanine has yielded more concordant results in the study of the kinetics of melanine formation by tyrosinase than any that have been employed up to the present time. Without going into details, this author (24) has shown that in the production of melanine from tyrosin, tyrosinase undoubtedly obeys the law of mass action, the departures therefrom observed during the later phases of the reaction being due to the fact that the activity of the ferment becomes more or less exhausted during the course of the reaction, this exhaustion being the more rapid the greater the concentration of the ferment or substrat; that is, the greater the velocity of the reaction.

THE ACTION OF TYROSINASE ON VARIOUS AMINO COMPOUNDS, ESPECIALLY THE PRODUCTS OF PROTEIN DEGRADATION.

It has been pointed out that laccase is not specific as an oxygen carrier, but that it can effect the oxidation of various easily oxidizable substances such as guaiacum, guaiacol, hydroquinone, phenolphthalin, etc. The question naturally suggests itself in this connection, Is the action of tyrosinase confined to tyrosin or can it likewise accomplish the oxidation of other aromatic amino compounds? The earlier workers in this field were doubtless inclined to look upon it as specific in its action on tyrosin. Thus vegetable tyrosinase (from *Russula delica*) was employed by Bougault (72) as a reagent for the detection of tyrosin in various animal products, and by Harlay (206) for the detection of tyrosin in the products of the pancreatic digestion of fibrin, and also in the products of the proteolysis in germinating grain. With the products of the pancreatic digestion of fibrin, tyrosinase gives a reddish brown color (tyrosin); with the products resulting from the peptic digestion of fibrin, Harlay found the extract of russula to give a green color. This indicates, of course, the absence of tyrosin, but it also indicates that tyrosinase can act upon substances other than tyrosin. During the last few years this conduct of tyrosinase toward a large number of aromatic amino compounds has been studied, including the optical isomers of tyrosin, and also the effect of various amino compounds and other substances on the action of tyrosinase on tyrosin. Thus it has been found by Bertrand and Rosenblatt (66) to act equally well upon racemic and laevo-tyrosin. They found the tyrosinase from *Russula quelctti* Fr., to give equal amounts of melanine with (d + l) and l-tyrosin, in a given time, without any separation of the racemic compound into its optically active components. According to Chodat and Staub (117) albumoses do not give a red color with tyrosinase. Such a coloration is produced by the action of tyrosinase on glycyl-tyrosin anhydride, indicating that possibly other peptids may give the reaction. In a continuation of their researches on the action of tyrosinase on the products of protein degradation, these authors (118) have observed that the oxidation of tyrosin by tyrosinase is diminished by certain amino acids, such as glycin, leucin, and alanin. They have found tyrosinase to act upon certain dipeptids, such as tyrosin anhydride, and glycyl-tyrosin anhydride, giving rise to yellow substances which do not become black, as does tyrosin itself. When, however, an amino acid, such as glycin, leucin, or alanin, is present, a red coloration similar to that resulting from tyrosin is obtained. Thus, a mixture of glycyl-tyrosin anhydride with glycin gives with tyrosinase a rose color, changing to bluish green; with alanin it gives a deeper red, and with leucin a deep brown color. Phenyl-alanin is not acted on by tyrosinase. These observers conclude, therefore, that the action of the ferment does not depend altogether upon the presence of a benzene nucleus in an amino acid. They have also found that tyrosinase acts readily on p-cresol, less readily on m-cresol, and still less readily on o-cresol. As a rule they have observed tyrosinase to act most readily on the homologues of phenol, in which the side chains occupy the para- position, and in this respect the ferment seems to differ essentially from Millon's reagent, which is apparently specific for benzene compounds containing one hydroxyl group, especially meta derivatives. According to Chodat and Staub (supra) the action of tyrosinase on p-cresol serves to distinguish the ferment from laccase. The addition of glycin or another amino acid greatly increases the rapidity of its action on p-cresol, giving rise to violet color which ultimately becomes blue with a reddish fluorescence. These authors conclude that tyrosinase may be employed as a reagent for tyrosin and that with the addition of amino acids, the ferment may also be employed to detect peptids containing tyrosin residues in the products of the digestion of protein.

Bertrand (62) in his recent researches on melanogenesis, has also studied the action of tyrosinase from wheat bran on various compounds analogous to tyrosin. With phenyl-alanin, phenyl-ethyl-amin, phenyl-methyl-amin, phenyl-amino-acetic acid, phenyl-propionic acid, phenyl-acetic acid, alanin and glycocoll, no coloration was observed. On the other hand, compounds containing phenolic hydroxyl were oxidized with the production of characteristic colors, as may be seen from the following table:

Name of compound.	Color produced with tyrosinase.
Tyrosin	Grenadine-red; then inky black.
p-hydroxy-phenyl-ethyl-amin	Grenadine-red; then olive-black.
p-hydroxy-phenyl-methyl-amin	Orange-yellow, orange-red, clear maroon.
p-hydroxy-phenyl-amin	Orange, mahogany-red, brown.
p-hydroxy-phenyl-propionic acid	Orange-yellow, grenadine-red, brown.
p-hydroxy-phenyl-acetic acid	Yellow, orange-yellow, brown.
p-hydroxy-benzoic acid	Rose, orange, yellow.
p-cresol	Yellow, orange, red.
phenol	Yellow, orange, red, brown.

He concludes, therefore, that only substances containing phenolic hydroxyl are oxidized by tyrosinase. He found that polypeptids are not colored exactly as is tyrosin, but become first yellow, then orange, and then mahogany-red, without the production of any precipitate. He concludes, therefore, that if in these polypeptids there were previous splitting into tyrosin and other products, one should obtain the same coloration as with tyrosin, since glycocoll in the proportion ordinarily found would not modify the action of the tyrosinase on tyrosin. In his opinion, it is necessary to separate the chromogen in pure condition in order to properly identify it by means of tyrosinase.

The action of tyrosinase from *Russula delica* on tyrosin, tyrosin-containing polypeptids, and certain other compounds, under various conditions, has also been studied by Abderhalden and Guggenheim.(¹) According to these authors glycocoll, d-alanin, d-valin, l-prolin, d-serin, d, l-iso-serin, l-phenyl-alanin, l-aspartic acid, and d-glutaminic acid are without effect on the action of tyrosinase on tyrosin, except in so far as they influence the rapidity of the development of the color. Aspartic and glutaminic acids were found to inhibit the action, as did also the other amino acids, especially if present in strong solution. The action of tyrosinase was also tried on the following substances: l- and d-tyrosin, di-iodotyrosin, l-phenyl-alanin, homogentisic acid, l-tryptophane, skatol, indol, l-prolin, and cystine. Of these, homogentisic acid and tryptophane were the only substances except tyrosin to show a color change with the ferment. On the other hand, polypeptids containing tyrosin residues were colored by tyrosinase, the color being modified to some extent by the nature of the amino acid combined with the tyrosin in the polypeptid. Halogen derivatives of the polypeptids were not acted upon. The action of tyrosinase on a polypeptid containing tyrosin was modified to some extent by various amino acids. Thus the action was greatly accelerated by l-prolin, whereas it was retarded by aspartic and glutaminic acids. Prolin was found to act especially energetically in augmenting the action of tyrosinase on glycyl-l-tyrosin anhydride. They also found tyrosinase to act on phenol, giving a brown color, and here again the color produced by the action of tyrosinase was modified by amino acids. Thus glycocoll and phenol gave a cochineal color, and prolin and phenol gave a violet reaction. These authors conclude that the character of the pigment resulting from the action of tyrosinase on tyrosin is dependent upon the combination in which the tyrosin exists. In the free state it is colored differently from what it is when in the anhydride or in the polypeptids. The amino acids when present apparently take part in the production of the pigment. In a later communication (2) these authors point out that tyrosinase acts rapidly on d-alanyl-l-tyrosin, and on l-leucyl-l-tyrosin. They also found it to act on adrenalin with the rapid production of a red color and ultimately dark red flocculi. It was also found to act on the three optical isomers of adrenalin with equal rapidity.

ON THE NATURE OF TYROSINASE.

Bach and Chodat (28) (see p. 118–120) have shown that laccase is composed of two distinct substances, an oxygenase—that is, a substance which forms a peroxide by taking up of oxygen and which is replaceable by hydrogen peroxide, —and a peroxidase, which activates this peroxide or the hydrogen peroxide added. According to this conception, the system, peroxidase + hydro-peroxide, is to all intents and purposes identical with the oxidases in its general behavior toward readily oxidizable substances. The question, therefore, naturally suggests itself in this connection, Is tyrosinase similarly constituted? In other words, Is this oxidase composed of a specific peroxidase and an oxygenase, and can other peroxides, such as hydrogen peroxide, take the part of the oxygenase in tyrosinase oxidations? Bach (21) has attempted to answer these questions. According to this observer, tyrosinase contains a peroxidase and an oxygenase, and it is to the former that it owes its specific power to oxidize tyrosin and similarly constituted substances, since hydrogen peroxide may be employed in the place of the oxygenase contained in tyrosinase in accomplishing the oxidation of tyrosin. Thus he observed that a fresh aqueous extract of young potato tubers rapidly oxidizes and blackens a solution of tyrosin, whereas if the expressed juice of finely ground new potatoes be allowed to stand for twenty-four hours with one-tenth of its volume of strong alcohol in order to remove mucilaginous substances, and the filtrate therefrom be mixed with four times its volume of absolute alcohol, there is obtained, after filtering and drying in vacuo over calcium chloride a dark-grayish mass, which on treatment with water dissolves only in part. After treatment with water and filtering, a perfectly clear and colorless solution is obtained, which shows strong peroxidase reactions, but weak oxygenase reactions, and which only acts upon tyrosin after standing from thirty-six to forty-eight hours. In other words, by the action of alcohol, the activity of the potato tyrosinase has been greatly weakened, a fact which is in harmony with Bertrand's earlier observations on the tyrosinase contained in *Russula delica*. According to Bach, it is weakened for the reason that the oxygenase moiety of the ferment has been destroyed by the alcohol. He therefore sought to restore it to its original activity by the addition of small amounts of hydrogen peroxide. As a matter of fact, the weak tyrosinase solutions which only oxidize the tyrosin after thirty-six to forty-eight hours, become dark brown in one hour after the addition of small amounts of hydrogen peroxide. Bach concludes, therefore, that the specific character of tyrosinase lies in the specific nature of its peroxidase.

On the other hand, R. Chodat and Staub ([118]) found that hydrogen peroxide not only did not accelerate the action of tyrosinase on tyrosin, but actually retarded it. As Bach pointed out in his first communication on the subject ([21]), however, tyrosinase is very sensitive to the action of hydrogen peroxide, and it is necessary to work with very dilute hydrogen peroxide in order to demonstrate the accelerating effect. Von Fürth and Jerusalem ([178]) have also observed that small amounts of hydrogen peroxide materially accelerate the action of tyrosinase, whereas with larger amounts of the peroxide the reaction is retarded.

More recently Bach ([23]) has been able to accelerate the oxidation of tyrosin by weak tyrosinase from *Russula delica* by means of hydrogen peroxide. As already pointed out under the preparation of tyrosinase (see p. 74), he prepared aqueous extracts of the ferment from three lots of the fungus (I, from fresh, unblemished fungi; II, from older, more or less damaged fungi, and III, from putrid fungi). Portions of these original extracts were diluted ten times with water, and 10 c. c. of the diluted extracts were mixed with 10 c. c. of a tyrosin solution containing 0.05 per cent of tyrosin and 0.04 per cent of sodium carbonate, and 30 c. c. of water added. After standing twenty-four hours, 1. c. c. of 10 per cent sulfuric acid was added, and each solution was titrated with 0.002 N potassium permanganate to complete decolorization. The quantities of permanganate required to decolorize, and the original colors resulting from the action of the tyrosinase, were as follows:

	Number of extract.		
	I.	II.	III.
Appearance before titration	Deep black; black sediment.	Violet black.	Dark brown.
Permanganate required .... c. c.	37.8	13.6	8.3

In order now to determine the effect of hydrogen peroxide on each of these extracts, similar experiments were carried out, except that to each solution 1 c. c. of 0.05 per cent solution of hydrogen peroxide was added to each of the tests. After standing twenty-four hours the three solutions were titrated with 0.002 N permanganate, with the following results:

	Number of extract.		
	I.	II.	III.
Permanganate required .... c. c.	37.3	26.7	23.2

It is evident from these results that the weaker the tyrosinase the greater the accelerating effect of hydrogen peroxide on its activity. After a few days extract No. I had become acid in reaction, and was slimy and brown and not in a condition to be filtered. In order, therefore, to neutralize the acid and coagulate the slimy material, the extract was treated with 10 grams of magnesium carbonate and filtered. The residue was then treated with 30 c. c. of water, in which, however, but little of the residue dissolved. Ten cubic centimeters of this solution were then mixed with an equal volume of the tyrosin solution. After twenty-four hours the mixture was entirely colorless. When, however, 0.5 c. c. of a 0.05 per cent solution of hydrogen peroxide was added, a tolerably rapid oxidation of the tyrosin occurred, and in the course of ten hours the mixture was colored black. It would seem, therefore, that by shaking the extract of the fungus with magnesium carbonate the peroxidase of tyrosinase can be separated from its oxygenase. A partial separation of the constituents of tyrosinase can also be accomplished by means of methyl alcohol. Thus, according to Bach (ibid.), 100 c. c. of the fungus extract were poured into 500 c. c. of strong methyl alcohol. The resulting precipitate was rapidly filtered, washed with methyl alcohol, and dried in vacuo over calcium chloride. The dry residue was rubbed up in a mortar with 100 c. c. of water, whereby only a small amount of the residue passed into solution. This mixture was then filtered and the filtrate tested toward tyrosin with and without hydrogen peroxide. While the solution containing hydrogen peroxide showed the characteristic blackening with tyrosin after twelve hours and required 17.6 c. c. of 0.002 N permanganate to decolorize it,[a] the test without hydrogen peroxide remained colorless for two whole days. Bach concludes, therefore, that at ordinary dilutions hydrogen peroxide exerts no influence on fresh, normal tyrosinase. As the result of certain changes in the tyrosinase, however, which may be brought about naturally or by artificial means, whereby the ferment becomes greatly weakened, its activity toward tyrosin may be greatly increased by dilute hydrogen peroxide. He is therefore of the opinion that the simplest view to take of the weakening of tyrosinase and its partial restoration by hydrogen peroxide is to refer it to the destruction of its unstable oxygenase.

In a more recent communication Bach (25) shows that it is impossible to oxidize tyrosin by the oxidase of *Lactarius vellereus*, and also that it is impossible to oxidize it by this peroxidase in the presence of hydrogen peroxide and a preparation of tyrosinase which had previously been destroyed by boiling.

It has also been shown by Chodat (116a) and also by Bach (21, 22) that ordinary peroxidase and hydrogen peroxide are without action on tyrosin. It would seem, therefore, that tyrosinase presents us with a case of specific ferment action connected in some way with the chemical constitution of the substance oxidized, and that while the two oxidases, laccase and tyrosinase, are similarly constituted in the sense that each contains a peroxidase and an oxygenase, and that in each case the oxygenase component may be replaced by hydrogen peroxide, the two enzymes differ in the specific character of their peroxidase constituent. The peroxidase of laccase is specific in the sense that while it can activate hydrogen peroxide toward guaiacum, hydroquinone, pyrogallol, phenolphthalin, etc., it can not activate it toward tyrosin, and while the peroxidase of tyrosinase can activate hydrogen peroxide toward tyrosin, and certain other amino compounds, it can not activate it toward the laccase reagents.

ANTI-TYROSINASE.

According to Gessard (182), the blood serum of a rabbit which has been inoculated with vegetable tyrosinase at successive intervals, retards the action of the ferment on tyrosin to a considerable extent, so that a long interval is required for the solution of the ferment and tyrosin to exhibit the succession of color changes ordinarily shown by a solution containing tyrosin and tyrosinase. This author reached the conclusion, therefore, that by the repeated injection of tyrosinase into an animal, there is developed in the blood of the animal receiving the injection an anti-body, to which he gave the name *anti-tyrosinase* (183). He observed further that as the result of the repeated injection of animal tyrosinase (from the ink sac of the cuttle fish) into an animal, such as the rabbit, the blood serum of the animal receiving such injections acquires the property of retarding the action of tyrosinase of the same origin. On the other hand, such serum was found to be without effect on vegetable tyrosinase. On the other hand, Von Fürth and Jerusalem (178) were unable to obtain any evidence of the formation of an anti-tyrosinase in the blood serum of rabbits following the injection of tyrosinase from the hemolymph of certain lepidoptera.

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[a] The excess of hydrogen peroxide remaining at the end of these experiments was in all cases removed by the addition of 1 c. c. of catalase solution. This quantity of catalase solution was sufficient to decompose 10 c. c. of a 1 per cent solution of hydrogen peroxide in one minute.

OXIDASES FROM VARIOUS SOURCES.

Since the earlier work of Bertrand and Bourquelot on laccase and tyrosinase, a number of oxidases have been obtained by various observers from different sources, to which special names ending in *-ase* have been given, usually to indicate something pertaining to the particular occurrence of the ferment, but in some cases referring to some peculiar chemical transformation which they can effect. Among these may be mentioned the following:

ŒNOXIDASE (OXIDASE OF WINE).

According to Cazeneuve (114, 115) and other French investigators, this is the oxidase which is responsible for the disease of wine known to French wine makers as *La Casse* or *Cassure*, as the result of which a red wine loses its characteristic color, due to the oxidation and sedimentation of its characteristic coloring matter. According to Martinand (287), this oxidase is present in the ripe grapes, whereas, according to Laborde (256), it is produced by a fungus, *Botrytis cinerea*, which grows freely on grapes and on wine must and is known commonly as the "sweet rot." It has also been found by Martinand (287) in other fruits beside the grape, such as the plum, pear, and apple. It has also been shown that under ordinary conditions the greater quantity of the ferment normally present in the fresh juice of the grape is lost or disappears during fermentation. In all probability it is carried down by the precipitates which go to form the wine stone. According to Martinand, it is destroyed in four minutes at 72° C., or even at 55° C., after an exposure of one and one-half hours. Bouffard and Semichon (71) have found it to be destroyed by very dilute sulfurous acid, 0.02 gram of sulfur dioxide per liter of enzyme. In many respects œnoxidase resembles laccase. It is worthy of note in this connection, however, that, according to Legatu (259), the disease of wine ordinarily ascribed to œnoxidase is in reality due primarily to the presence of ferrous salts in quantities above the normal. These are oxidized to ferric salts and then precipitated by the tannin, the precipitate carrying down the coloring matter of the wine. According to this author the only part played by the œnoxidase is that it assists in the rapid oxidation of the ferrous salts, and hence may participate to that extent in accomplishing the changes already described.

MALOXIDASE (APPLE OXIDASE).

The color changes occurring in fruits like the apple, pear, peach, etc., as a result of a wound or cut in the fruit or an abrasion of the skin are familiar to everyone. It is also known that no such changes of color occur if the fruit be previously heated to about the temperature of boiling water. The effect of heat in preventing these changes is also seen to good advantage in various sorts of artificially and naturally dried fruits, especially dried apples. Apples which have been dried in the sun by natural processes are brown in color in consequence of the oxidation of tannin by an oxidizing ferment, maloxidase, contained in the fruit (*see* Lindet (271, 272)). On the other hand, evaporated apples which have been dried artificially at higher temperatures, and in some instances even exposed to the action of sulfur dioxide, are white, for the reason that the oxidase has been destroyed. The juice of the apple has been found to blue guaiacum and to oxidize hydroquinone and pyrogallol. In this connection it has been pointed out by Kastle and Shedd (247) that those vegetable tissues which readily oxidize guaiacum and phenolphthalin rapidly turn brown or reddish in color when their freshly cut surfaces are exposed to the air, whereas the tissues of those plants which do not oxidize these reagents do not turn brown or red on exposure to the air. In other words, the oxidases present in the raw fruit are responsible for both phenomena.

According to Lindet (271, 272) the oxidase of the apple and the tannin upon which it acts are stored in different cells, and hence it is only when these are brought together by actual rupture of the cells that we have those color changes occurring which are characteristic of the bruised or macerated fruit. On the other hand, Kastle and Loevenhart (244) have pointed out that, on the assumption that the oxidizing ferments are of the nature of peroxides, the oxidase (peroxide) is not present as such in the intact cell, but only its precursor, viz., an autoxidizable substance which, when it comes into contact with the air through the rupture of the tissue or cell, unites with the oxygen to produce the peroxide or the so-called oxidizing ferment, or according to the views of Bach and Chodat (28) the precursor of the oxygenase portion of the ferment. Hence the tannin and the substance destined to become the oxidase or a part thereof as the result of the action of oxygen could exist together in the same cell, or, for that matter, even in the same solution, and yet no oxidation or no coloring take place until the cell had been ruptured and air admitted. It seems highly probable that the so-called maloxidase and Bertrand's laccase are the same.

SPERMASE.

This oxidase has been detected by Grüss (201) in the embryo of the barley by means of Wurster's reagent (tetramethyl-para-phenylene diamin). If a grain of barley be cut in two along the greater length of the grain and the cut surface pressed against moist "tetra" paper, a violet color develops on the exposed surface of the embryo, while the endosperm remains colorless. On the other hand, if the grain be heated to 55° C. for fifteen minutes the embryo shows no color with "tetra" paper, indicating that the ferment has been destroyed. The ferment scarcely colors guaiacum, and hence, according to Grüss, should not be confused with laccase. He is of the opinion that it plays a part in the morphological and physiological changes which accompany the production of malt. Thus, he has shown that in the kiln-drying of very moist malt, without ventilation, the oxidase is destroyed (201).

OXIDIN (*Boutroux*).

According to Mégé-Mourier (293, 294, 295), the characteristic color of brown bread is produced during panification by a substance of ferment-like nature, to which he gave the name "*Cerealin.*" Afterwards Boutroux (92) showed that these changes of color result from the action of an oxidase (laccase), to which, for reasons already indicated, he gave the name "*Oxidin.*" Quite recently Bertrand and Mutermilch (65) have obtained tyrosinase from wheat bran and have reached the conclusion that the changes of color observed in bread during baking are due, in part at least, to the action of this ferment. It is quite likely, therefore, that Mégé-Mourier's *Cerealin* and Boutroux's *Oxidin* are merely mixtures of laccase and tyrosinase.

SCHINOXIDASE.

Sarthou (357, 359) obtained this oxidase from the latex of *Schinus molle* by the usual method. An aqueous extract of the latex of this plant blues guaiacum and oxidizes the phenols, such as hydroquinone, resorcin, and pyrogallol, and transforms potassium ferrocyanide into potassium ferricyanide with the absorption of oxygen. The ferment differs from Bertrand's preparations of laccase chiefly by the fact that the ash is rich in iron, but contains no manganese. According to Sarthou (358), this iron exists in the active ferment in organic combination, and to this the ferment owes its oxidizing power.

OLEASE.

Tolomei (424) has observed that fresh olives gradually undergo fermentation when exposed to the air, with the production of acetic and other fatty acids and the evolution of carbon dioxide. According to this author these changes are brought about by the action of an oxidase to which he has given the name "Olease." This ferment has also been found by Tolomei in the olive oil itself, and to its action are due those changes which take place when the oil becomes rancid. The acids produced by its action serve to gradually weaken the ferment.

PURPURASE.

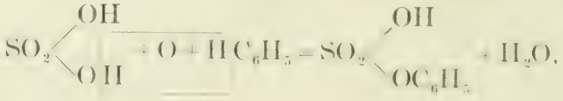
One of the most interesting and picturesque changes attributable to oxidizing ferments is that described by Dubois (148), which results in the production of a purple pigment in the mollusc, *Murex brandaris*. According to this author the pigment glands of these gasteropods are analogous to the photogenic (luminous) organs of *Pholas dactylus*, the principal difference being that while the fixation of oxygen in the latter gives out its energy in the form of light, the latter absorbs luminous vibrations with the production of a pigment. In other words, the changes occurring in the production of the pigments of molluscs are also photochemical. Dubois observed that on extracting the pigment gland with absolute alcohol the chromogen contained in the gland passed into solution. The alcoholic solution of the chromogenic substance was concentrated on the water bath and the concentrated solution used to impregnate test papers for the purpose of experimentation. The residue of the gland remaining after extraction with cold absolute alcohol was extracted first with chloroform water, and the residue was finally macerated with glycerin, all of these operations being carried out in the dark or in the feeble light of the dark room. On adding a drop of the glycerin extract of the gland to the test paper containing the chromogenic substance of the gland and moistening with water and exposing to sunlight, a purple color gradually developed on the paper, the intensity of which depended on the time of exposure and the intensity of the light. The glycerin extract alone is not colored on exposure to the light, and it loses its activity when heated to 120° C. in the autoclave. Three factors are therefore concerned in the development of the purple pigment in molluscs, (1) a chromogenic substance soluble in alcohol, (2) a ferment, and (3) light. According to Dubois, the chromogenic substances (*les substances chromogeniques*) concerned in these changes consist of a definite, crystallizable substance, the chromogen proper, which has been described by Letellier (268) (*see* footnote to Dubois's article), and a ferment. To the former Dubois gave the name "purpurin," and to the latter "purpurase." No special evidence has been brought forward by Dubois, or by anyone else for that matter, to indicate that purpurase is an oxidizing ferment. Indeed, its conduct toward the ordinary oxidase reagents seems never to have been investigated. However, the changes resulting in the production of the pigment in the pigment gland of *Murex brandaris*, in which it evidently participates, present many close analogies to those oxidations which are accomplished by oxidases, and hence certain authors have been led to put purpurase in the class of oxidizing ferments.

LUCIFERASE.

The production of light by animals and plants has been the subject of many researches. In this connection it was long ago pointed out by Dubois (115, 117) that the active agent of light production in animals and plants is a substance having the general properties of a ferment. This enzyme he has called "luciferase." According to Dubois (116), the transformation resulting in the production of light in animals and plants takes place under the influence of life, water, and a suitable temperature. From his earlier researches on the subject he concluded that physiological light is not the result of a combustion, nor even of a slow oxidation, but a direct chemical act (*mais directe*). In his later investigations, however, he claims to have shown that the fixation of oxygen is necessary, the oxidation taking place not directly but indirectly, and for the most part, at least, through the action of luciferase as an intermediary, which thus conducts itself like an oxidizing ferment. In this connection he observed that the luminous organs of the *Lampyridæ* and also the eggs contained in the ovaries of the females give a beautiful blue color with tincture of guaiacum. So, also, the filtered extract of the luminous mucus from the body of a dead fish, prepared with chloroform water, gave a similar reaction. Dubois (117) concludes, therefore, that the photogenic substances concerned in the production of physiological light are "luciferin" and "luciferase." In other words, the light generated by various life forms is the result of a chemical change, probably an oxidation, brought about by the action of luciferase on luciferin. My own experience with the firefly native to the Central and Middle Western States, *Luciola pennsylvanica*, is that the aqueous extracts of the luminous organs do not show the guaiacum reaction directly, but only after the addition of hydrogen peroxide. The whole subject requires further investigation.

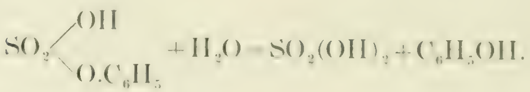
ALDEHYDASE (SALICYLASE).

We have seen that Schoenbein (384), especially as the result of his work on the oxygen-carrying power of blood, recognized the importance of oxygen carriers for oxidation processes occurring in the animal organism. Traube (427) also believed that a complete analogy exists between the respiratory changes occurring in muscle and the process of slow combustion. His theory of muscular activity was based upon the assumption that the muscle fiber contains a vital, oxidizing (*verwesungs*) ferment (*see* Traube (425), p. 107), which carries oxygen from the blood to the oxidizable substances contained in the muscle fluids, without the ferment itself suffering any destruction. These or similar views were also shared by other distinguished observers. Thus, according to Claud Bernard (47) the respiration of tissue is not a direct combustion, but an indirect oxidation accomplished by chemical agents of the nature of ferments. Some few observations were made by Schützenberger (393) on the oxidation of certain substances in the animal organism. It remained for Schmiedeberg to greatly extend our earlier knowledge of such processes. Thus, as the result of their investigation of the formation of hippuric acid in the animal organism, Bunge and Schmiedeberg (106) came to the conclusion that the red blood corpuscles play an essential rôle in the formation of hippuric acid in the living kidney, for the reason, possibly, as these observers surmised, that it plays the part of an oxygen carrier. From these observations Schmiedeberg himself (366) was naturally led to the study of those oxidations which in the animal organism might result in the formation of hippuric acid. He arrived at the conclusion that the principal changes occurring in the animal organism are splittings (hydrolysis), oxidation, and synthesis, and, as he proved in these researches, the two latter processes frequently go hand in hand. It was shown that benzyl alcohol was oxidized by blood to only a slight extent, and almost equally well by a solution of sodium carbonate, but not at all in pure water. Similar results were obtained with salicylic aldehyde. On the other hand, by the action of oxygenated blood in the tissue of the kidney and lung both of these compounds are oxidized a thousand times more rapidly than they are by blood alone or by a solution of sodium carbonate. From this it follows that conditions exist in the tissues whereby an increased activity is conferred on the oxygen of the blood, in that it is rendered active or gotten into the nascent state, or some change is effected in the oxidizable substance, whereby it becomes more easily oxidizable. This increased activity on the part of the oxygen may be explained as a result of the action of (1) an exciter analogous to platinum-black, or (2) of readily combustible substances in the tissues which have the power of decomposing the oxygen molecule, as a result of which not only would they themselves become oxidized, but other substances as well. This last was an application of Hoppe-Seyler's theory of oxidation (1878). On the other hand, Schmiedeberg observed that these aromatic compounds are more rapidly oxidized than phosphorus. This, he says, is evident from the fact that in phosphorus poisoning in man following the administration of 0.1 to 0.2 gram of the substance, some of the phosphorus apparently remained in the body in unaltered condition after death. Hence he distinguishes between synthetic oxidations and those like the oxidation of phorphorus, and points out that it is only substances containing hydrogen that lend themselves to such oxidations as the former. He concludes also that in the apparent activation of oxygen in such synthetic oxidations the living tissue acts not upon the oxygen molecule, but upon the oxidizable substance; otherwise it would oxidize the phosphorus as readily as the benzyl alcohol or salicylic aldehyde. Essentially similar views have recently been advanced by Mathews (289) in order to account for the oxidation of the sugars (see footnote, p. 56). Schmiedeberg also pointed out that all of these oxidation processes have this in common, viz., that the final product of the oxidation occurs in the urine in the form of a conjugated compound in which it is paired with sulfuric or glycuronic acid, or with glycol. Hence the production of phenol in the organism following the administration of benzene may result from the following changes:

(*1*) 

$$\mathrm{SO_2}\left\langle\begin{matrix}\mathrm{OH}\\ \mathrm{OH}\end{matrix}\right. + \mathrm{O} + \mathrm{H\,C_6H_5} = \mathrm{SO_2}\left\langle\begin{matrix}\mathrm{OH}\\ \mathrm{OC_6H_5}\end{matrix}\right. + \mathrm{H_2O},$$

and

(*2*) 

$$\mathrm{SO_2}\left\langle\begin{matrix}\mathrm{OH}\\ \mathrm{O.C_6H_5}\end{matrix}\right. + \mathrm{H_2O} = \mathrm{SO_2(OH)_2} + \mathrm{C_6H_5OH}.$$

See also Baumann and Herter (40).

As a matter of fact, Schmiedeberg found that a dog which had received 24 grams of benzene, in eight doses in twenty-four hours, excreted 1.6907 grams of phenol, of which 1.1005 grams were found in combination with sulfuric acid.

Several years later this subject was reinvestigated by Jacquet (222), who showed, first, that the blood alone does not possess the power of accomplishing the oxidation of such substances as benzyl alcohol and salicylic aldehyde; second, that certain animal tissues or cell-free extracts thereof in contact with blood or atmospheric oxygen have the power of accomplishing the oxidation of these substances; third, that while such extracts lose their oxidizing power on boiling, their oxidizing powers are not destroyed by carbolic acid, quinine, or by freezing, or by precipitation with alcohol and re-solution in water. He arrived at the conclusion, therefore, that oxidations in the animal organism are brought about by ferments or enzymes.

Further studies on the oxidizing ferments were made by Pohl (329) with the view of determining whether the several oxidizing ferments described by various authors as occurring in animal and plant tissues are the same or different enzymes. He reached the conclusion that two essentially different kinds of oxidations occur in living tissues and that these are brought about by at least two distinctly different ferments, one of which accelerates the oxidation of fatty (formic) and aromatic aldehydes, and the second brings about such oxidations as the production of indophenol-blue from an alkaline solution of a-naphthol and para-phenylene diamin. He found further that certain plant extracts have the power of accomplishing the indophenol reaction, but are powerless to oxidize formic or salicylic aldehyde or mannite. An aqueous solution of tannin needles proved to be especially active. In this connection he also showed that amygdalin gives the indophenol reaction apparently independently of the action of any ferment.

On the other hand, Spitzer (406) arrived at the conclusion that glycolysis (the disappearance of sugar in the blood) is only a special case of oxidation by blood and animal tissues, and that the glycolytic ferment of Lepine is probably identical with the oxidizing ferments in tissues capable of oxidizing aromatic alcohols and aldehydes. In support of these conclusions he cited, first, an observation by Salkowski (356) to the effect that under certain conditions blood can oxidize salicylic aldehyde, and, secondly, some observations of his own to the effect that those tissues which can rapidly oxidize alcohols and aldehydes can also destroy glucose. The results obtained in subsequent researches tend to confirm him (*see* Spitzer (407)) in the belief that the oxidizing ferments, especially of the animal organism, are substances of the same nature, viz., nucleoproteids, and that certain of these owe their activity as oxygen-exciters to the organic iron which they contain.

On the other hand, Abelous and Biarnes (7, 8) observed that certain globulins have the power to oxidize guaiacum, but are without action on salicylic aldehyde, and, as already pointed out, Raudnitz (338) proved that the substance in milk which causes the bluing of guaiacum in the presence of hydrogen peroxide is not identical with that which catalyzes the hydrogen peroxide. It was also pointed out by Lepine (262) and also by Jacoby (224) that the glycolytic ferment differs from the oxidizing ferment oxidizing salicylic aldehyde in several respects, especially in regard to the effect of temperature on the two ferments, the glycolytic ferment being destroyed at 58° C., whereas the oxidizing ferment under consideration is only destroyed at 100° C. This last observation was confirmed by Jacoby (224). From these and other observations Jacoby (225) came to the conclusion that several oxidizing ferments occur in the liver, so that according to this author it seemed no longer advisable to refer to the ferment capable of oxidizing salicylic aldehyde merely as an oxidation ferment. He therefore adopted Bertrand's nomenclature in the naming of these substances, designating as *aldehydase* the ferment or ferments of the liver found to be capable of oxidizing aldehydes, and giving the name *salicylase* to the particular ferment concerned in the oxidation of salicylic aldehyde (*see* Jacoby (225)).

In order to obtain a water-clear solution of the ferment having powerful oxidizing powers, Jacoby (225) recommends the following method of preparation: Fresh beef liver is put through a sausage machine and then rubbed up with quartz sand, and the paste thus obtained mixed with some distilled water. Toluene is added and the mixture allowed to stand several hours with vigorous shaking; the mass is then filtered. The dark, clear liquid thus obtained is then saturated with ammonium sulfate to the extent of 25 per cent and the fluid rendered weakly alkaline with sodium carbonate, so that after saturation with ammonium sulfate the liquid generally smells of ammonia. In about twenty-four hours a small amount of precipitate is produced in the liquid, which is then filtered off. The filtrate is then saturated to the extent of 33⅓ per cent with common salt, and after twenty-four hours the precipitate is again removed by filtration. The clear, dark-colored liquid is then saturated to the extent of 60 per cent with ammonium sulfate, with the result that a heavy precipitate is obtained which requires about twenty-four hours to settle out completely. This precipitate, which contains the aldehydase, is then filtered off, washed with 60 per cent ammonium sulfate solution, and then taken up with distilled water, in which it only partially dissolves. After several hours the aqueous solution is filtered, and 95 per cent alcohol gradually added to the clear filtrate, until a good filterable precipitate is obtained. As a general thing sufficient 95 per cent alcohol has to be added to bring the concentration of the alcohol in the whole liquid up to about 30 per cent. The solution containing the precipitate is then filtered. The precipitate is then extracted five or six times with distilled water containing a few drops of dilute soda solution, and the extracts united. As a rule the ferment is most completely extracted by allowing the finely divided precipitate to stand over night with water. The bright clear liquid thus obtained is still found to contain protein. It is then made faintly alkaline with soda and uranyl acetate added until a filterable precipitate is obtained, which is then handled in precisely the same way as the alcoholic precipitate. There is thus obtained finally a water-clear liquid which vigorously oxidizes salicylic aldehyde and which now gives none of the protein reactions.

The ferment thus obtained is soluble in water and, as has been pointed out, can be salted out with ammonium sulfate. It is practically nondialyzable. In spite of its great solubility in water, the ferment is not removed from the liver by intensive washing out of the circulatory system with 0.7 per cent salt solution. At a pressure of six atmospheres the ferment passes through the Chamberland filter. The aldehydase was found by Jacoby to be perceptibly soluble in 20 per cent alcohol, but insoluble in concentrations equal to or greater than 30 per cent. It is also precipitated from its solutions by tannin. With dilute solutions of the ferment the Millon and biuret tests were found to be negative. Aldehydase was found to lose its oxidizing power on boiling or by treatment with small amounts of free acid or alkali. Toward salts it conducts itself much like a globulin, and yet at the concentration at which it vigorously oxidizes salicylic aldehyde it does not give the protein reactions.

That aldehydase is a ferment is indicated by the fact that it is not consumed in the oxidations which it can accomplish, but can react with fresh quantities of the oxidizable substance. Thus Jacoby allowed a clear extract of liver to act upon salicylic aldehyde for forty-eight hours, at the end of which time small portions of the liquid gave an excellent test for salicylic acid. The main portion of the liquid was then dialyzed against water for three days and the ferment was then salted out with ammonium sulfate and the precipitate dissolved in water. Two-thirds of this solution alone gave no test for salicylic acid after seventy-two hours' digestion, whereas another portion digested with salicylic aldehyde gave an abundant test for salicylic acid.

In addition to the above-mentioned researches, aldehydase has formed the subject of numerous investigations during the past fifteen years, among which may be mentioned those by Salkowski and his coworkers, Abelous and Biarnes, Medwedew, and others. The occurrence and distribution of the ferment in various animal tissues has been studied by Salkowski and by Salkowski and Yamagiwa, and also by Abelous and Biarnes. In oxidizing power toward salicylic aldehyde, Salkowski and Yamagiwa (355) found certain animal organs to stand in the following order:

Liver = 100. 0
Spleen = 80. 4
Kidney = 15. 5
Pancreas = 2. 0
Muscle = 1. 0,

whereas Abelous and Biarnes (5) arranged the tissues in the following order with regard to their activity toward salicylic aldehyde:

1. Spleen.
2. Lung.
3. Liver.
4. Thyroid.
5. Kidney.
6. Thymus.
7. Suprarenal.
8. Testicle.

According to these authors muscle, pancreas, and brain are incapable of oxidizing salicylic aldehyde. Medwedew (291) studied the kinetics of the oxidation of salicylic aldehyde by extracts of various organs and has found that the quantity of salicylic aldehyde oxidized is proportional to the square of the concentration of the ferment, and inversely proportional to the square root of the concentration of the aldehyde.

One of the most interesting facts disclosed by these investigations is that the oxidation of the aldehyde by aldehydase is independent of the presence of atmospheric oxygen. Thus in certain of his experiments Medwedew (292) found the following amounts of salicylic acid to be produced (1) during aeration, and (2) in the absence of air:

No. of experiment.	Amount of salicylic acid found, in milligrams.	
	(1) Aerated.	(2) In absence of air.
1	85	83
2	118	115
3	80	78

It will be observed that the differences between the several amounts of salicylic acid formed in the two cases are very small and are probably within the limits of experimental error, and yet in experiment No. 3 (1) was subject to a continuous current of air for 16 hours, whereas (2) remained without aeration, and portion (2) of experiment No. 2 was kept in a hermetically sealed vessel. Similarly it has been shown by Abelous and Aloy (4) that the oxidation of salicylic acid by various organs proceeds more rapidly in a vacuum than in the presence of air. In fact, according to these authors, liberal supplies of free oxygen diminish the rate of oxidation very considerably and may even suppress it altogether. These authors are of the opinion, therefore, that in such oxidations as take place in a vacuum the oxygen necessary for the oxidation is furnished by certain oxygen compounds which are dissociated by the oxidizing ferment. In other words, such processes as the oxidation of salicylic aldehyde under the influence of aldehydase partake of the nature of anaerobic fermentations, and, as is well known, the oxygen required for the latter processes is furnished by various oxygen-containing substances participating in the fermentation. In this connection it is interesting to recall that Traube provided for such cases in his general theory of fermentation. According to this distinguished observer the oxygen of water might under certain conditions be transferred to the oxidizable substance by means of a ferment, and in the fermentation of a compound like sugar the oxygen present in one part of the molecule might be transferred to another part of the molecule, thereby giving rise, after splitting, to one substance richer in oxygen than the original, and to another poorer in this element. Such a change is met with in the breaking up of glucose into carbon dioxide and alcohol by yeast. It is interesting to note in this connection that similar changes have been met with among inorganic substances; thus, when an aqueous solution of phosphorous acid is heated, phosphoric acid and phosphine are produced—

$$4H_3PO_3 = 3H_3PO_4 + PH_3$$

Whether water actually participates in such changes is at present unknown. In my opinion these simple processes would warrant further investigation from the point of view of anaerobic fermentation.

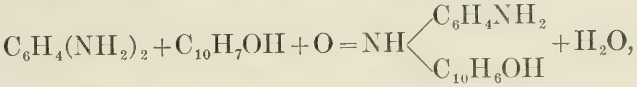
In this connection Abelous and Gerard (9,10) have shown that extracts of various animal tissues have the power of reducing nitrates to nitrites. So also it was afterwards found by Abelous (3) that the juice of the potato can also reduce nitrates, but is incapable of oxidizing salicylic aldehyde. On adding a small amount of potassium chlorate, however, the aldehyde is oxidized at the same time that the chlorate is reduced. This property of the juice is not lost on boiling. In some respects these reactions are similar to those investigated by Kastle and Elvove (243). These authors also observed that the aqueous extract of the potato has the power of reducing nitrates to nitrites and that in the presence of certain readily oxidizable organic compounds, such as formic aldehyde and benzyl alcohol, the quantities of nitrite formed are greatly increased. On boiling, the aqueous extract of the potato loses its power to reduce nitrates even in the presence of readily oxidizable substances. All of these facts point to the presence in the potato and in animal tissues of certain ferments which have the power of effecting the transfer of oxygen from a substance rich in this element, such as the chlorate or nitrate, to an easily oxidizable substance. According to Abelous (3) these are the oxido-reducing ferments.

Dony-Hénault and Mlle. J. van Duuren (141) have recently investigated the oxidation of salicylic aldehyde by means of aqueous extracts of calf's liver. According to these observers the methods for the estimation of salicylic acid are far from exact. In their own work they adopted the method of Elion of estimating the salicylic acid formed during the oxidation by means of bromine. They have also arrived at the conclusion that the salicylic acid appears to combine in part at least with certain substances present in the extracts and hence would be likely to escape detection in most of the methods. They agree with Abelous and Aloy (1) that the acid is only produced rapidly in the absence of oxygen. The quantity of salicylic acid produced in a given time also varies with the concentration of the aldehyde and the amount of extract employed, the former being the preponderating factor in determining the velocity of the reaction. They observed, further, that the oxidizing power of extracts of the liver diminishes spontaneously when such extracts are left to themselves, especially at higher temperatures. According to these authors, the results thus far reached in the study of this oxidation do not warrant the conclusion that the oxidation in question is accomplished by a ferment acting in the absence of air. About all that can be said is that a certain amount of oxidizing material is present, but this being limited the amount of salicylic aldehyde oxidized by a given quantity of extract is also limited, while the oxidase is a true catalyst, probably of a mineral nature, and as such ought to be able to accomplish the oxidation of practically unlimited quantities of material. According to these authors the presence of such substances has not been proven in such animal extracts as those under immediate consideration, nor is it necessary to assume their presence in order to account for the phenomena observed.

THE INDOPHENOL OXIDASE: THE RÖHMANN-SPITZER REAGENT.

In his study of the oxygen requirements of the organism it was shown by Ehrlich(157), in 1885, that the intravenous injection of α-naphthol and para-phenylene diamin results in the formation of indophenol in the tissues of the animal. So also Würster (158) showed that di- and tetramethyl-para-phenylene diamin are reagents for active oxygen, and that in the animal organism these substances are converted into blue coloring matters. Acting upon these suggestions, Röhmann and Spitzer(151) have made use of a reagent consisting of equal amounts of α-naphthol and para-phenylene diamin, in a dilute solution of sodium carbonate (three molecules of sodium carbonate to one molecule of each of the organic compounds), for the investigation of the oxidizing power of the various animal tissues. Various other related substances can be employed in the preparation of the reagent; thus dimethyl-para-phenylene diamin(153) can be used instead of para-phenylene diamin. They also showed that other coloring matters result from the oxidation of suitable mixtures by the action of various animal tissues. Thus they succeeded in showing that certain indamines like toluene-blue result from the oxidation of meta-toluene diamin and para-phenylene diamin, and Bindschedler's green from dimethyl-para-phenylene diamin and dimethylanilin, and that the synthesis of various eurhodins may be accomplished in the same manner. An easy method of demonstrating such reactions consists in placing a small amount of an animal tissue, such as the liver, on a test paper which has been prepared with the two chromogenic components of the dye. If, for example, a small amount of fresh liver be placed upon a test paper which has been saturated with a sodium carbonate solution of α-naphthol and para-phenylene diamin, the place on the paper occupied by the tissue is soon deeply colored, whereas the other portions of the paper show only a slight coloration. According to these authors, the change in question is represented by the following equations:

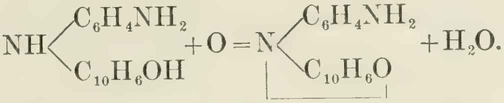
1.



$$C_6H_4(NH_2)_2 + C_{10}H_7OH + O = NH\left<\begin{array}{l}C_6H_4NH_2\\C_{10}H_6OH\end{array}\right. + H_2O,$$

and

2.



$$NH\left<\begin{array}{l}C_6H_4NH_2\\C_{10}H_6OH\end{array}\right. + O = N\left<\begin{array}{l}C_6H_4NH_2\\C_{10}H_6O\end{array}\right. + H_2O.$$

Thus one atom of oxygen goes to effect the synthesis of the leuco compound and a second atom to effect the oxidation of the leuco compound to the coloring matter. When the solution of the chromogens is allowed to stand in contact with atmospheric oxygen, the coloring matter is formed only slowly in the presence of alkalies; when the animal tissue is added the process is greatly accelerated. It was shown by these authors that these oxidations are most readily accomplished by extracts of cells, the body fluids and secretions being comparatively inactive. In this respect the change is analogous to the destruction of glucose, a change which is readily accomplished by extracts of red and white blood cells in physiological salt solution, but not by blood serum. They reached the conclusion, therefore, that the oxygen exciters responsible for these oxidations are contained only in the cells. That such is the case is evident also from the conduct of such extracts and secretions toward guaiacum and hydrogen peroxide. Extracts of red blood cells blue a mixture of guaiacum and hydrogen peroxide instantaneously, whereas the blood serum causes no change of color, or if it acts at all it does so very feebly. While they knew nothing of the particular cell components responsible for these changes, they seemed to think that their observations furnished evidence, hitherto wanting, of a single oxidizing ferment. By precipitating the macerates of fresh organs with alcohol and washing the precipitate thus obtained with alcohol and ether, they obtained dry powders which retained their oxidizing powers for a year, whereas the alcohol extracts were found to be inactive. A comparison of the different organs showed that they activated oxygen toward these reagents with different degrees of intensity. As a rule they were found to be energetic oxygen carriers, accomplishing these oxidations more rapidly than they were brought about by palladium foil or palladium-hydrogen. Finally, these authors called attention to the general similarity between the synthesis of indophenol and similar dyes with that of bromphenol cystein, which, according to Baumann, results from the administration of brom-benzene to dogs. They arrived at the conclusion therefore that the cells of animal tissues contain substances which have the power of activating molecular oxygen, whereby it can accomplish the oxidation of substances not ordinarily directly oxidizable (*dysoxydabler Stoffe*). They protest, however, against the supposition that the oxidation of all difficultly oxidizable substances in the organism depends on the action of oxygen-carriers.

The indophenol oxidase is by no means confined to animal tissues. Mention has already been made of the work of Pohl (329) on this subject. This author found that many plant tissues react strongly to the Röhmann-Spitzer reagent. Especially is this the case with tannin needles. He also pointed out that the reaction is also brought about by amygdalin, apparently altogether independently of the action of an oxidase. It is possible, of course, that his amygdalin may have been contaminated with a vegetable oxidase. Rey-Pailhade (312) also found the indophenol oxidase to be widely distributed among plants, and according to this author yeast reacts feebly to the Röhmann-Spitzer reagent. Abelous and Biarnes (5) have determined the oxidizing power of various tissues from the same and different animal species, colorimetrically, by means of the Röhmann-Spitzer reagent. According to these authors, the tissues of the frog and rabbit, the latter killed by bleeding, stand in the following order with regard to oxidizing power:

Frog.	Rabbit.
Lung.	Spleen.
Liver.	Lung and thyroid.
Kidney.	Liver and kidney.
Testicle.	Pancreas and suprarenals.
Brain.	Ovary.
Muscle.	Brain.
	Muscle.

It will be observed that there is general agreement between these findings and those of the same authors and also of Salkowski with regard to the relative oxidizing power of the various animal tissues toward salicylic aldehyde (see pp. 97–98).

One of the most interesting facts revealed by these determinations is the slight oxidizing power of muscle and nerve tissue as compared with that of other animal tissues, and, as pointed out by Abelous and Biarnes (5), this is all the more remarkable when it is borne in mind that the muscular system is the seat of very powerful exothermic reactions. In this connection it has been observed by Kastle (240) that extracts of muscle and brain greatly retard the oxidation of an alkaline solution of phenolphthalin by blood, and it may be, as suggested by him in explanation of his own results, that muscle and nerve tissues may be unusually rich in powerful reducing agents which would interfere with the oxidation of all extraneous oxidizable material. The occurrence and distribution of various intra-cellular ferments, including the indophenol oxidase, has been studied by Rosell (352) with the following results. In the table the presence of the ferment is indicated by the sign (+) and its absence by the sign (−).

Intra-cellular ferments in beef tissues (*Rosell*).

Organ.	Aldehydase.	Indophenol oxidase.	Catalase.	Trypsin.	Pepsin.
Pancreas	−	+	+	+	−
Salivary glands	+	+	+	+	−
Lymph glands	+	−	+	+	−
Spleen	+	+	+	+	+
Bone marrow	−	+	+	+	+
Thymus	+	+	+	+	−
Lacteal glands	−	−	+	+	−
Muscle	−	−	+	+	−
Lung	+	−	+	−	+
Brain	+	−	+	−	−
Suprarenals	+	−	+	+	−
Testicle	+	−	+	+	−
Kidney	+	−	+	−	−

The presence of the guaiacum-oxidase could not be established with certainty.

THE PURIN OXIDASES (XANTHIN-OXIDASE).

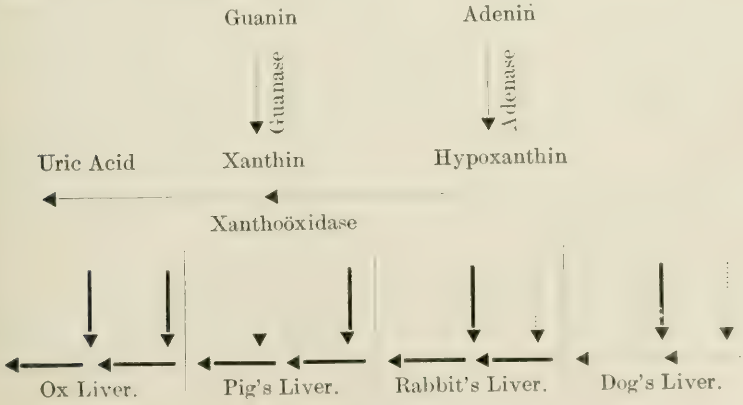
It has long been known that on a flesh diet alone the ratio of urea to uric acid, $\frac{\text{urea}}{\text{uric acid}}$, in the urine is fairly constant, varying from 45 to 65. On the other hand it was pointed out by von Noorden (312) that on different diets it is subject to enormous variations, even in the same individual. Thus in some of his cases it ranged from 23.2 to 122.4. So also by feeding milk, peptone, and vegetable proteid, Camerer (108) was able to double the total nitrogen secreted without causing any increase in the output of uric acid. On the other hand it was pointed out by Kossel (253) that those foodstuffs which cause an increase in the excretion of uric acid contain much larger amounts of hypoxanthin than human muscles. Thus the muscles of the hen and pigeon contained over 0.1 of hypoxanthin per 100 grams of moist muscle, whereas the muscles of man contained only 0.039 to 0.048 gram. It was afterwards shown by Weintraud (145) that following a diet rich in nucleins, as for example, the calf's thymus, the uric acid in the urine is considerably increased, whereas according to Hess and Schmoll (211) a similar increase in the uric acid does not follow a nuclein-free proteid diet.

On the other hand it is of interest to note in this connection that uric acid itself can be destroyed to a certain extent in the organism, and that following the administration of uric acid by the mouth in men and dogs only a small part of it is recoverable from the urine (*see* Minkowski, 302). It has also been shown by Minkowski that hypoxanthin causes a distinct increase in the output of uric acid, and that meat extract which contains considerable amounts of this substance has a similar influence on the uric acid excretion. That certain purin bases are formed by the decomposition (autolysis) of the nucleins was shown by Horbaczewski (218, 219). This author allowed a mixture of spleen pulp and water to stand at 50° C. From this slightly putrid mixture he was able to isolate large amounts of xanthin and hypoxanthin, whereas from the fresh extract of spleen only traces of these purins were obtainable. After shaking the slightly putrid mixture with air he obtained no xanthin or hypoxanthin, but only uric acid. Similar results were obtained by adding blood or hydrogen peroxide to the slightly putrid spleen pulp. It is evident from these results that the nucleins of the spleen are the precursors of the purins.

On repeating Horbaczewski's work, Spitzer (409) showed that uric acid is formed in aqueous extracts of spleen pulp through which is passing a current of air and in which putrefaction is prevented by chloroform or thymol, and that contrary to the opinion of Horbaczewski, xanthin and hypoxanthin can be readily converted into uric acid by the action of a current of air on an aqueous extract of spleen containing these substances at 50° C. Similar results were obtained with the liver, but other organs, such as the kidney, pancreas, and thymus, were found to be much less active in their power to accomplish this oxidation. Similar results were obtained by Wilner (152), who arrived at the conclusion that in the liver, not only of birds but also of certain mammals, there are produced large amounts of uric acid. He also showed that the addition of hypoxanthin to liver extracts causes a great increase in the quantity of uric acid obtainable from the extract.

Evidently, therefore, the oxidation of xanthin and hypoxanthin is accomplished *in vivo* and *in vitro* through the instrumentality of an oxidizing ferment. This ferment has been made the subject of an exhaustive investigation by Burian (107) and named by him *xanthin-oxidase*. By following Spitzer's method and using one part of macerated liver to two parts of chloroform water he was able to obtain clear extracts of xanthin-oxidase containing only very small amounts of purin bases. On digestion in a current of air these extracts alone gave practically no uric acid. They were found, however, to oxidize xanthin and hypoxanthin rapidly in a current of air. By means of these preparations of xanthin-oxidase he was able to confirm the previous observations of Spitzer and Wilner and to show that, as is the case with many ferments, the conversion of xanthin and hypoxanthin under the influence of the ferment is a reaction of the first order. He showed also that the xanthin-oxidase is not consumed in the process, but is capable of oxidizing fresh quantities of xanthin and hypoxanthin in the presence of oxygen. Hence it would seem to be a true ferment. This is evident from the fact that no noteworthy falling off in the coefficient of the velocity of the reaction was observable.

On the other hand, the reverse process, viz, the reduction of uric acid to other purin bodies does not take place to any noticeable extent. He is of the opinion therefore that xanthin-oxidase is responsible for the production of uric acid from purin bases in the living animal. Contrary to the observations of Wilner (452) he found that in the absence of purin bases, tartronic and dialuric acids cause no production of uric acid with his extracts of xanthin-oxidase. He concludes therefore that these substances can act only by accelerating the action of the xanthin-oxidase on purin bodies already present in the liver extracts. Neither can xanthin-oxidase convert guanin or adenin directly into uric acid, but, as has been found by Jones and Austrian (230, 231), these substances must first be desamidized by the action of guanase and adenase, whereby they are converted into xanthin and hypoxanthin, respectively, before their oxidation into uric acid can occur. The occurrence and distribution of these ferments have been exhaustively studied by Jones and Austrian (230, 231) and also by Schittenhelm (365) and others. A very good idea of their distribution in the liver of various animals is afforded by the following diagram, which is given in certain of Jones's original contributions to the subject:



In the above diagram the solid black lines indicate the presence of the particular ferment to which it appertains, whereas the dotted lines indicate the absence of the ferment: the first diagram explains the four that follow.

It is evident from these results that the production of uric acid from xanthin or hypoxanthin takes place directly as the result of the combined action of the xanthin-oxidase and molecular oxygen, whereas it can only be formed from guanin and adenin by previous desamidization through the action of guanase and adenase, respectively, which belong to the group of hydrolytic ferments.

Reference has been made repeatedly to the wide distribution in nature, especially in the plant kingdom, of oxidases of the type of laccase. Whether these represent merely special occurrences of laccase or whether they are really distinctly different oxidases can not be determined in the present state of our knowledge. So far as one can judge from the experimental evidence now at hand it would seem probable, however, that all of these guaiacum-bluing vegetable ferments are pratically indentical with laccase, and it is doubtful if there is much to be gained by the introduction of these new names, which after all only serve to tell something with regard to a special occurrence of the ferment without throwing any light on differences in properties, if such exist. On the other hand, Sarthou (357, 358) is inclined to the opinion that the oxidases are distinct substances which can not replace one another in the changes which they bring about.

Irrespective of these differences of opinion, however, the following additional facts pertaining to their occurrence are of interest.

Aso found oxidases in tea (15) and in kaki fruit (14); Loew (277) found them in tobacco, and Carles (110) in valerian; they have been found in the grapevine by Cornu (123), in plants of the genus *Spiræa* by Beijerinck (41), and in the vanilla bean by Lecomte (257). According to Breaudat (95) indigo-producing plants contain two ferments, one a hydrolytic enzyme and the other an oxidase; the latter in the presence of alkali transforms indigo-white into indigo-blue. On the other hand Bergtheil (46) was unable to find an oxidase in the indigo plant. Bourquelot ([illegible]) has proved the presence of oxidases in certain medicinal preparations and in certain gums such as gum arabic, gum senegal, etc. Lepinois ([illegible]) found an oxidase in aconite and belladonna, and Vadam ([illegible]) found an oxidase in hellebore. Similarly oxidases have been found in oleaginous grains by Mazé (290) and in digitalis by Brissemoret and Joanne (98).

As is evident from what has already been set forth in this monograph, the oxidases occur abundantly in the higher fungi. In addition to this they have been found in different yeasts, such as *Saccharomyces ellipsoideus*, *S. cerevisiæ*, and *S. apicultus*. According to

Tolomei (^{423}), Effront (^{156}), and Buchner (^{103}) the oxidases of these yeasts oxidize guaiacum and are concerned in the production of the bouquet of certain wines. To these also are attributable the darkening in color of zymase (expressed yeast juice) on exposure to the air, and also the rise in temperature produced when large amounts of yeast cells are exposed to the air. These observations by Tolomei and Effront on the occurrence of oxidases in yeasts have been confirmed by Grüss (^{202}), only this author found these oxidases to be without action on guaiacum or hydroquinone. On the other hand, like his spermase, they were found to be capable of oxidizing tetra-methyl-para-phenylene diamin, and other amino compounds, such as phenylhydrazin. Hence according to Grüss the yeast oxidase belongs to the class of amino-oxidases.

Issajew (^{221}) has also investigated the oxidases of yeast and has confirmed the work of the earlier observers mentioned. He found that the oxidase in yeast oxidizes both the easily oxidizable substances which the yeast contains and also other easily oxidizable substances, such as the polyphenols, and that the upper yeast (*Oberhefe*) contains, as one might naturally expect, more oxidase than the under yeast (*Unterhefe*).

Various bacteria, such as the bacillus of malignant pustules (Dietrich and Liebermeister (^{139}), and the colon bacillus (Roux (^{353})), have been found to contain oxidases. The oxidase of the latter was found to oxidize hydroquinone, gelatin, peptone, etc., with the production of a brown pigment, and to be active only in the presence of oxygen.

Chapter III.

THE PEROXIDASES AND CATALASES.

In addition to the oxidases, two other classes of enzymes are more or less immediately concerned in the oxidation processes occurring in the plant and animal. These are the peroxidases and catalases. These have been found to have even a wider distribution among living cells and tissues than the oxidases. They were called by Bourquelot (83) the "indirect oxidizing ferments." It was known to Schoenbein (383) that many substances of animal and vegetable origin which in themselves are incapable of bluing guaiacum, acquire this property when mixed with hydrogen peroxide. In other words, just as certain extracts and tissues have the power of rendering active the oxygen of the air, so certain others possess the property of rendering active the oxygen of hydrogen peroxide, thereby enabling this compound to effect certain oxidations which ordinarily it is incapable of bringing about. Thus on adding a small amount of extract of malt to a mixture of guaiacum and hydrogen peroxide the mixture rapidly becomes blue in color. He also made the interesting observation that many plant and animal tissues and the extracts thereof and many hydrolytic ferments have the power of effecting the decomposition of hydrogen peroxide into water and oxygen. He seems to have regarded the power to render active the oxygen of hydrogen peroxide and the power to catalyze this substance not as specific activities of any given substances but as properties pertaining to the ferments as a class. In other words, the power to oxidize guaiacum by means of hydrogen peroxide and the power to decompose the latter substance into water and oxygen were looked upon by him simply as properties of diastase, emulsin, myrosin, and other ferments. This view prevailed for a long time and was shared by others. Thus, according to Spitzer (407), the power to decompose hydrogen peroxide is a measure of the oxidizing power of various animal tissues. It was afterwards shown by Raudnitz[a] (338, 339), however, that the substance in milk which gives the guaiacum reaction with hydrogen peroxide is really different from the other substances present in milk, in that it conducts itself differently toward various precipitants; and Jacobson ([illegible]) pointed out that the power of any given tissue or ferment to decompose hydrogen peroxide can be destroyed by certain degrees of heat and by certain poisons which have no action on the other ferments present in the preparations. Similar conclusions were also reached by Loew (278) in his work on catalase. (*See also* Bourquelot (83).) Hence we have come to regard the peroxidases and catalases as distinctly specific enzymes. Apparently, therefore, three distinctly different sets of substances are concerned in vital oxidations: First, the oxidases, by means of which the oxygen of the air is rendered sufficiently active to effect the oxidation of guaiacum and other oxidizable substances directly; second, the peroxidases which render active the oxygen of hydrogen peroxide and other peroxides (including the oxygenases); and third, the catalases, which decompose hydrogen peroxide into water and molecular oxygen, without, apparently, being able to activate the oxygen of the peroxide toward oxidizable substances.

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[a] Raudnitz ([illegible]) called the catalase of milk a superoxydase, and the guaiacum-bluing ferment a globulin oxydase.

Schoenbein (383) included the peroxidases and catalases among his oxygen-carriers and catalysts (*Sauerstofferreger* and *Sauerstoffueberträger*) without apparently assigning particular names to these particular groups of carriers. Later, those substances which induce the oxidation of guaiacum and similar reagents through the agency of hydrogen peroxide and which lose this property on boiling, were called by Bourquelot (83) "indirect oxidizing ferments" (*ferments oxydants indirect*), and in 1898 Linossier (273) gave them the name of peroxidases as signifying those substances whose function it is to decompose hydrogen peroxide and other analogous peroxides and thereby induce oxidations by means of these peroxides. The term "catalase" was proposed by Loew (278) for those ferments which decompose hydrogen peroxide into water and molecular oxygen without apparently being able to activate the oxygen of the peroxide toward readily oxidizable substances.

The peroxidases and catalases seem to be even more widely distributed in various living tissues of the plant and animal than the oxidases. To such an extent is this the case that the properties of these substances might almost be turned to account as a general chemical test for vital activity. It can certainly be said of any living tissue or organ that it is dead when it fails to show the reactions of the peroxidases and catalases. Thus Brocq-Rosseu and Gain (100), in their observations on the duration of the peroxidases in grains, found that all seeds which retained their germinating power contained peroxidases, and in corn peroxidases were recognized by these observers (99) in samples of the grain over two hundred years old. On the other hand, it was observed by these authors that the peroxidase activity outlasts the power to germinate by a hundred years. In other words, it would appear that peroxidase activity is one of the most characteristic and persistent properties of living material.

SOURCES OF PEROXIDASE AND METHODS FOR ITS PREPARATION.

Among plants the peroxidases are almost universally distributed. There are but few, if any, plant tissues which do not give peroxidase reactions at some stage of their growth and development. So, likewise, they are widely distributed in animal tissues and secretions; saliva, milk, pus, etc., show typical peroxidase reactions. By means of certain of the very sensitive peroxidase reagents employed by Kastle and Porch (246) in their study of the peroxidase reaction of milk, Doctor Roberts, working in this laboratory, has been able to stain a large number of the leucocytes in fresh blood smears, indicating that in the blood the peroxidase proper is confined to the white cells. Thus with a reagent containing cresol, para-phenylene diamin and hydrogen peroxide, the leucocytes are stained blue.

By precipitation with alcohol, Linossier (273) prepared a peroxidase, free from oxidase, from pus. In many instances plant peroxidases may be obtained free from oxidases by heating the aqueous extract to 70° C., at which temperature the oxidase is destroyed. Aso (16) has separated plant peroxidases from oxidases by fractional precipitation with alcohol, in which solvent the oxidases are soluble to a considerable extent.

As sources of peroxidase, Bach and Chodat (27) have employed the pumpkin (*Kürbisfrüchten*) and the root of the horse-radish (*Meerrettigwurzel*). In order to obtain the peroxidase, these authors proceed as follows: Two kilograms of finely grated horse-radish root are allowed to stand several hours at ordinary temperature in order to completely hydrolyze all glucosides that may be present. The mass is then digested for four to five days with 80 per cent alcohol in order to dissolve the essential oils. The reddish alcoholic extract is then poured off, the residue is washed with 80 per cent alcohol, pressed, and extracted with about 8 liters of 40 per cent alcohol. The 40 per cent alcoholic extract, which shows strong peroxidase reactions, is then reduced to small volume at 30° C. in vacuo, filtered, and absolute alcohol added until a turbidity is produced. The white precipitate thus produced is dissolved in a small amount of water, again precipitated with absolute alcohol, and dried in vacuo. In this way a yellowish white gummy mass is obtained which is very soluble in water and readily soluble in 40 per cent alcohol. As ordinarily obtained these peroxidase preparations strongly reduce Fehling's solution. By re-solution in water and re-precipitation with alcohol, peroxidase preparations may be obtained which no longer reduce Fehling's solution. The purest peroxidase preparations obtained by this method give ammonia and substances having the odor of pyridin on heating with caustic soda, but failed to show the ordinary reactions for proteid. On heating to boiling, solutions of the peroxidase were found to lose their activity; on standing, however, such boiled solutions regain their activity. That such is the case was first observed by Woods (463) in his study of the peroxidase of tobacco, and has been accounted for by Woods, and also by Aso (16), upon the supposition that the peroxidase is regenerated from zymogens which are more stable towards heat and various reagents than the ferment itself. Bach and Chodat (27) find that a second heating destroys the peroxidase altogether; so also an alcoholic solution of the ferment is destroyed by heating to the temperature of boiling alcohol. The interval required for the destruction of the enzyme has been found to vary with the concentration of its solution. Thus when diluted with twenty times its volume of water a given specimen which in its original dilution required eighteen minutes in boiling water for complete destruction, required but three minutes at this temperature when thus diluted. According to Bach (19) the peroxidase is a single enzyme, whose function is to activate hydrogen peroxide in the oxidation of substances containing labile hydrogen.

Bach and Tscherniack (32) have given detailed directions for the purification of peroxidase. No essential differences have been observed between the conduct of crude peroxidase and that of the purest preparations. The pure preparations were found to contain 6 per cent of ash; this ash was found to be iron-free, but to contain aluminium and manganese. Bach and Chodat (27) found that peroxidase, the chief constituent of oxidase, contains nitrogen and gives the pyrrol reaction. They conclude, however, that it is not a proteid since neither the raw product nor the purified enzyme show the protein reactions.

According to Bach and Chodat (27) the action of peroxidase is specific in that it strongly activates hydrogen peroxide and other peroxides in a large number of oxidation processes, such as the oxidation of pyrogallol, gallic acid, anilin, dimethylanilin, etc. They observed further that while it strongly activates small amounts of hydrogen peroxide, it is destroyed by larger amounts of the peroxide (*see also* Schoenbein (379), p. 474), and also that in the absence of hydrogen peroxide or a similar substance the peroxidase has no oxidizing power (*see also* Linossier (273)). Decidedly the most interesting observations recorded by these observers is that the peroxidase not only activates hydrogen peroxide and the peroxides resulting from the slow oxidation of various organic compounds such as ether, alcohol, and the essential oils, but that it also has the power of increasing the oxidizing power of the oxidases. As elsewhere pointed out, Moore and Whitley (306) argue from this that the oxidase is nothing more than a mixture of peroxidase and an unstable, naturally occurring peroxide (*see also* Bach and Chodat (28)).

Quantitative studies of the oxidation of pyrogallol by hydrogen peroxide and a peroxidase have also been made by Bach and Chodat (31) with the following results:

(1)

[Pyrogallol, 1 gram; hydrogen peroxide, 0.1 gram; peroxidase, from 0.01 to 0.1 gram, in 50 c. c.]

Peroxidase	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08
Purpurogallin	0.021	0.042	0.066	0.083	0.102	0.123	0.145	0.166

(2)

[Pyrogallol, 1 gram; peroxidase, 0.1 gram; hydrogen peroxide, from 0.01 to 0.1 gram.]

Peroxide	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08
Purpurogallin	0.020	0.042	0.060	0.078	0.099	0.121	0.142	0.168

(3)

[Peroxidase, 0.1 gram; hydrogen peroxide, 0.1 gram; pyrogallol, 1 to 4 grams.]

Pyrogallol	1.0	1.5	2.0	3.0	4.0
Purpurogallin	0.168	0.205	0.203	0.208	0.202

It would seem therefore that the amount of pyrogallol oxidized under these conditions is proportional to both the quantity of the peroxidase and of the hydrogen peroxide, but independent of the quantity of pyrogallol present, provided that the latter is in excess of the quantity capable of being oxidized within the given time by the several amounts of the peroxidase and hydrogen peroxide employed.

NATURE AND MODE OF ACTION OF THE OXIDASES, PEROXIDASES AND CATALASES, AND THE CHEMICAL RELATIONSHIPS EXISTING AMONG THESE SUBSTANCES.

The question still remains to be considered as to how these substances act as oxygen carriers. We have seen that according to Schoenbein they react by ozonizing the oxygen of the air, and in the case of the peroxidases, by converting hydrogen peroxide, an antozonide, into an ozonide, after the manner of lead acetate. On the other hand, according to Bach (18) the oxidases are substances having a special aptitude for forming peroxides. Similar views have been advanced by Kastle and Loevenhart (24). Thus according to the peroxide theory of oxidation, when molecular oxygen finds itself in contact with the complex autoxidizable substances contained in the plant cell, it combines with the same in much the same way that it unites with rubidium or benzaldehyde. There is produced under these circumstances a complex unstable peroxide which, in turn, can give up a part, if not all of its oxygen, to any oxidizable substance or acceptor that may happen to be present, or in the event that no other oxidizable substance is present, it may oxidize a part of itself.

In this manner we account for the oxidation of guaiacum, potassium iodide, and hydroquinon by atmospheric oxygen through the intermediate action of the vegetable oxidase. This theory also enables us to understand the instability of these substances.

Diagrammatically, these changes may be represented as follows:

$$(1) \qquad A + O_2 \overset{\text{(Oxidase)}}{=} AO_2,$$

and

$$(2) \qquad \overset{\text{(Oxidase)}}{AO_2 +} B = AO + BO,$$

or

$$(3) \qquad AO_2 + 2B = A + 2BO,$$

and

$$(4) \qquad AO_2 + A = 2AO.$$

In this connection see also Engler and Weisberg (164), supra.

In the above equations *A* is the autoxidizable substance or substances contained in the plant or animal which is the immediate precursor of the oxidase. AO_2 is the oxidase resulting from the union of *A* with molecular oxygen, and *B* is the substance oxidized by the oxidase, as, for example, guaiacum, phenolphthalin, or hydroquinon. If the change proceeds as indicated in equation (2), both the precursor of the oxidase and the second oxidizable substance (the oxidase reagent) will be completly oxidized, and the oxidase will not be regenerated as the result of the oxidation, but will be destroyed. On the other hand, if the oxidation proceeds according to equation (3), the precursor of the oxidase will be regenerated, and at the end of the process will be in a condition to again enter into the cycle of changes indicated in equations (1) and (3). Finally, it is conceivable that the oxidase may oxidize a second part of *A*, in which case, as indicated by equation (4), both the precursor of the oxidase and the oxidase itself will be destroyed. As a matter of fact, it is known that solutions of oxidases gradually lose their oxidizing powers; this is readily intelligible in the light of equations (1), (2), and (4), especially in view of the fact that all living tissues and extracts contain powerful reducing substances which in the processes under consideration would function as *B* in equation (2).

In support of the view that the oxidases are really peroxides, we may cite the following observations of Bach and Chodat (26): The fresh sap of the *Lathræa squamaria*, which contains an oxidase, was subjected to the action of a current of air and a 1 per cent solution of barium hydroxide was added drop by drop. A precipitate is formed under these circumstances which is found to contain barium, and when decomposed by sulfuric acid the resulting solution gave a blue coloration with potassium iodide and starch, but no reaction with titanic acid. Nitrites were also shown to be absent. Hence, according to these authors, the separation of the iodine from the potassium iodide could only have been brought about by an acylhydroperoxide. A similar experiment carried out on a specimen of the *Lathræa squamaria* sap which had lost its power to blue guaiacum gave a completely negative result. Hence the peroxide formation with the active sap depends on the presence of the oxidase, and leads to the belief that the oxidase itself is of a peroxide nature, or that it at least contains a peroxide as one of its constituents.

Schoenbein, in his paper on the catalytic action of organic materials (peroxidases and catalases) and their distribution in the plant and animal kingdoms (388), explains the activating and catalyzing power of such substances on the supposition that, like lead acetate, they convert hydrogen peroxide (an antozonide) into an ozonide (like lead peroxide), and that under the influence of the latter the former is actively decomposed with the production of water and molecular oxygen. Thus he proved that when lead acetate is added to a solution of hydrogen peroxide, lead peroxide (an ozonide) is formed, under the influence of which the hydrogen peroxide is actively decomposed; and that if lead acetate be added to hydrogen peroxide solution containing guaiacum, the latter is oxidized at the same time that a part of the hydrogen peroxide is decomposed, for the reason that the lead peroxide oxidizes the guaiacum at the same time that it decomposes the hydrogen peroxide. According to Schoenbein, therefore, what we now know as the peroxidases are those substances occurring in the secretions and tissues of animals and plants which have the power of ozonizing hydrogen peroxide or converting it into an ozonide. The catalysis or decomposition of the hydrogen peroxide he looked upon as a secondary phenomenon resulting from the action of the ozonide thus formed upon the hydrogen peroxide remaining unchanged. Lepinois (263) conceives that the decomposition of hydrogen peroxide takes place in such a way that where only a part of the oxygen is set free it is fixed by the guaiacum or guaiacol.

From their study of the hydrogen peroxide-guaiacum reaction, Kastle and Loevenhart (244) arrived at the conclusion that the peroxidases are substances which are capable of reacting with hydrogen peroxide to form peroxides, which are more vigorous oxidizing agents than hydrogen peroxide itself. This view regarding the nature of these substances has been concurred in by Bach (29). The decomposition of hydrogen peroxide and the mechanism of oxidations by means of this substance has formed the subject of a still further investigation by Loevenhart and Kastle (275). It is now known that hydrogen peroxide undergoes spontaneous decomposition into water and molecular oxygen; it is also known that it can effect the oxidation of many substances directly, such as formic aldehyde and sulfurous acid, without the intervention of an oxygen carrier. It is also a matter of common observation that its decomposition into water and molecular oxygen is greatly accelerated by various catalysts, such as spongy platinum, lead peroxide, manganese dioxide, etc., and also that its oxidizing powers are greatly increased by these same substances. It has also been established through the researches of Schoenbein (383) and through some observations of Loevenhart and Kastle (275) that, with but few exceptions, those substances which can bring about the decomposition of hydrogen peroxide catalytically can also greatly increase its oxidizing power; and in proportion as a substance is able to decompose the peroxide, so also can it accelerate oxidations by this substance to a corresponding degree. A good catalyst has been found to be a good oxygen carrier and, vice versa, a poor catalyst is a poor carrier. Finely divided platinum decomposes hydrogen peroxide very rapidly; it has been found also to be a powerful oxygen carrier. On the other hand, sugar charcoal decomposes hydrogen peroxide very slowly; it is a very poor oxygen carrier. It is evident, therefore, that oxidation by hydrogen peroxide in the presence of a carrier and its catalytic decomposition go often, if not always, hand in hand, so that the latter process, as has been pointed out by Spitzer (407), is often a measure of the former. It would seem, therefore, that all of these phenomena, widely differing as they appear at first sight, are in reality closely correlated and referable to a common cause, namely, the tendency on the part of the hydrogen peroxide to unite directly with oxidizable substances, forming thereby either a peroxide or some other complex unstable holoxide derivative which tends to part with its oxygen more easily than the hydrogen peroxide itself. This derivative, therefore, readily oxidizes some other substance to which one of its compounds stands in the relation of an oxygen carrier, or it gives up molecular oxygen, or both.

It is important for this assumption, of course, that such higher oxides and unstable addition-products of hydrogen peroxide with other substances should actually exist. In reality, we have abundant proof of their existence. In fact, as may be seen from the following, many such compounds are known. Thus in 1878, Schöne (388, 389) isolated a number of compounds of hydrogen peroxide with the alkalis and alkaline earths, such as the following:

$BaO_2.H_2O_2$, or H_2BaO_4;
$K_2O_2.2H_2O_2$, or $H_4K_2O_6$;
$Na_2O_2.2H_2O_2$, or $H_4Na_2O_6$.

All of these compounds were found by this observer to be very unstable and are obtainable in a state of purity only at very low temperatures. He therefore explained the catalytic decomposition of hydrogen peroxide by alkalis on the assumption that these hydrogen peroxide addition-products with the alkalis are first produced, thus:

$$2R\text{OH} + 3H_2O_2 = H_4R_2O_6 + 2H_2O,$$

and that these substances then undergo the following transformations:

$$(1) \qquad R_2H_4O_6 = R_2O_4 + 2H_2O;$$
$$(2) \qquad R_2O_4 + H_2O = 2R\text{OH} + O_3.$$

He was led to assume the intermediate production of such peroxides as R_2O_4 for the reason that the compounds such as $H_4K_2O_6$ turned yellow during decomposition. The work of Schöne on these complex addition-products of hydrogen peroxide has been confirmed by the later researches of Forcrand (173), who has obtained evidence not only of the existence of such compounds as H_2BaO_4 and H_2CaO_4, but also of still more complex derivatives such as $CaO_2.10H_2O_2$, and Moissan (301) from his investigation of the unstable blue compound of chromic anhydride and hydrogen peroxide, reached the conclusion that it must be represented by the formula H_2CrO_5, or $CrO_3.H_2O_2$.

Similarly, in order to explain the great acceleration caused by small amounts of molybdic and tungstic acids in the oxidation of hydriodic acid by hydrogen peroxide, Brode (191) has been led to assume the formation of permolybdic and pertungstic acids as the result of the action of the peroxide on the catalyzer, while nine years previously Cammerer ([illegible]) had obtained permolybdic acid, $2MoO_3.H_2O.H_2O_2$, and pertungstic acid, $WO_3.H_2O.H_2O_2$, by boiling the ordinary acids with hydrogen peroxide, and still more recently Pissarjewsky and Mellikoff (225) and also Muthmann and Nagel (208) have obtained similar acids and their salts, some of the latter being very unstable and even explosive. According to Job (226) cerous salts act as oxygen-carriers in the presence of hydrogen peroxide, probably through the alternate formation and decomposition of cerium peroxide.

Petrenko (219) also, while studying the action of hydrogen peroxide on sodium arsenate, obtained a compound having the composition $Na_9As_3O_{17}.21H_2O$. This he found to be a hydrogen peroxide derivative having the following constitution: $3Na_3AsO_4.5H_2O_2.16H_2O$. So also Kazanesky ([illegible]) obtained the compounds $K_2CO_3.2H_2O_2.12H_2O$, and $K_2CO_3.3H_2O_2$, and similarly Staedel ([illegible]) found that on adding a 90 to 95 per cent solution of aqueous hydrogen peroxide to a concentrated solution of cadmium chloride, a compound was obtained which crystallized in silky needles containing 23 per cent of hydrogen peroxide, the theory for $CdCl_2.2H_2O_2$ being 27.09 per cent.

Jones and his co-workers ([232], [233]) have recently investigated the lowering of the freezing point of certain salts and acids in aqueous hydrogen peroxide as a solvent. In discussing the abnormal lowering of the freezing point of certain of these salts, such as potassium nitrate, in this solvent, these observers make this significant statement: "The most probable explanation of the above results, all things considered, seems to be that there is union between the molecules of the salts investigated and the molecule of hydrogen peroxide."

According to Manchot ([234]) the apparent reductions by means of hydrogen peroxide are in reality due to the formation of more highly oxidized primary products, which ultimately break down into final products which are less highly oxidized than the hydrogen peroxide itself. (*See also* Kastle and Loevenhart.)

There seems to be abundant evidence, therefore, that hydrogen peroxide is not only able to form peroxides by double decomposition, but that it also frequently combines directly with various substances to form complex unstable addition products, and that oxidations by means of this substance are often greatly accelerated and its decomposition greatly hastened by the formation and subsequent decomposition of these complex substances. It would seem logical, therefore, to look upon the peroxidases as substances capable of forming unstable peroxides from hydrogen peroxide by double decomposition, or of combining directly with hydrogen peroxide to form unstable holoxide (Traube) or moloxide (Engler) derivatives, possessing greater powers of oxidation than hydrogen peroxide.

Thus, if we designate the peroxidase by P, and the second oxidizable substance by B, the oxidations effected by the peroxidase might be represented diagrammatically as follows:

$$\begin{gathered} P + H_2O_2 = PO_2 + H_2O, \\ \text{and } PO_2 + B = PO + BO, \\ \text{or } PO_2 + 2B = P + 2BO, \\[1em] \text{or } P + H_2O_2 = H_2PO_2, \\ \text{and } H_2PO_2 + B = P + BO + H_2O. \end{gathered}$$

As already pointed out, these views respecting the mode of action of the peroxidases are shared by Bach, who has observed that when a mixture of a peroxidase and hydrogen peroxide is allowed to stand for some time both disappear from the solution, a fact which indicates the mutual interaction of these two substances. According to Bach and Chodat ([27]) the peroxidases exert not the slightest oxidizing action in the absence of the peroxide. On the other hand, they found that it can activate not only hydrogen peroxide but also organic peroxides, such as ethyl-hydroperoxide, $C_2H_5O_2H$. Further, they have shown that the system (peroxidase + peroxide) accomplishes precisely the same kind of oxidations as are accomplished by the oxidases, and that between the two sorts of oxidizing agents there is the closest sort of agreement, and in this connection they have made a number of interesting observations which would seem to indicate that the vegetable oxidases are not single enzymes but mixtures of peroxidases and peroxide-forming substances, to which they have given the name "oxygenase."

THE OXIDASE A MIXTURE OF PEROXIDASE AND OXYGENASE.

As early as 1898, Linossier ([273]), in discussing the function of the peroxidases, stated that the oxidases fix the oxygen of the air, forming peroxides which are destroyed by the peroxidases as fast as they are formed.

In the course of their investigations on the "Function of Peroxides in the Living Cell" Bach and Chodat ([37]) found that by the fractional precipitation of an aqueous solution of the oxidase of a fungus, *Lactarius vellereus*, by alcohol, two fractions could be obtained possessing markedly different properties. The first of these is almost insoluble in 40 per cent alcohol and was found to have the properties of a weak oxidase. The second substance is soluble in 40 per cent alcohol but insoluble in pure alcohol, and has no oxidizing powers. This second substance, however, was found to impart great activity to hydrogen peroxide as an oxidizing agent, and not only has it the power of conferring activity upon hydrogen peroxide, but also upon organic peroxides, such as ethyl hydro-peroxide, etc., and what is even more interesting, it was also found to impart activity to the weak oxidase composing the first fraction obtained in the precipitation of the *Lactarius* extract. In a recent paper these authors ([41]) give the following interesting results of their experiments with these substances:

Pyrogallol.	Horse-radish peroxidase solution.	Lactarius peroxidase solution.	Lactarius oxygenase	Oxygen absorbed.	Carbon dioxide evolved.
	c. c.	*c. c.*	*Gram.*	*c. c.*	*c. c.*
1 gram.	15	....	....	0.5	0.1
1 gram.	....	....	0.059	3.1	1.1
1 gram.	15	....	0.059	9.9	5.5
1 gram.	....	15	....	0.2	0.0
1 gram.	....	....	0.05	3.1	1.1
1 gram.	....	15	0.05	11.0	6.8

These results show conclusively that the oxidizing power of the oxygenases is greatly increased by the peroxidases in general, and still more greatly increased by the peroxidase from the same source as the oxygenase itself.

According to Bach and Chodat (28), therefore, the oxygenases are of the nature of substituted peroxides. They are exceedingly unstable and have, as compared with the peroxidases, only a limited distribution in the vegetable kingdom. On the other hand, the peroxidases are characterized by great stability. Thus the peroxidase of the horse-radish root is not completely destroyed by a single boiling of its solution. The peroxidases occur in practically every plant thus far examined for them, and they have likewise been found widely distributed in the tissues and secretions of animals, such as the leucocytes, milk, saliva, etc. Like the oxidases, the peroxidases have been found to contain manganese, and to this element Bach and Chodat (31) ascribe their activity. They are also disposed to regard them as true ferments, although they gradually lose their activity and ultimately disappear as the result of the oxidations which they bring about.

It would seem, therefore, that it is not the oxidase but rather the peroxidase which is the most important agent in plant and animal oxidations, inasmuch as it would render active the oxygen occurring in any peroxide combination whatsoever, whether it be hydrogen peroxide, an organic peroxide, or an oxygenase.

Indeed, according to Moore and Whitley (306) the peroxidases are the only true ferments participating in biological oxidations. According to these authors, Bach's so-called oxygenase is merely an unstable peroxide resulting from the action of the oxygen of the air on some readily oxidizable substance contained in the plant or animal tissue. From their standpoint the only essential differences between a tissue showing oxidase reactions and one showing only peroxidase reactions is that the former contains, in addition to the peroxidase, a store of naturally formed peroxides, which are very unstable toward heat, whereas the latter contains only the ferment (peroxidase). In this connection it was long ago pointed out by Bach (18), and also by Kastle and Loevenhart (244), that the oxidizing ferments (oxidases) were not true ferments for the reason that they present many close resemblances to the organic peroxides, and for the further reason that they are not true oxygen-catalysts in the sense of being unable to accomplish the oxidation of practically unlimited amounts of oxidizable material. In the writer's opinion, therefore, the objections which have been recently urged against the prevailing conceptions regarding the oxygenases are well taken. It should be borne in mind, however, that the precursors of the oxygenases are unstable toward heat and hence possess, to some degree at least, the characteristics of biologically active substances.

THE OXYGEN-CATALYSTS OF THE BLOOD.

Certain oxidases, such as aldehydase and the glycolytic enzyme, peroxidases and catalases have been found in the blood, and also certain more stable oxygen-catalysts, such as hemoglobin and its iron-containing derivatives, and in the case of certain of the invertebrates, hemocyanin, the oxidizing powers of which are not destroyed upon boiling. In this connection it is interesting to note that all of the oxygen carriers of the blood of whatever nature are contained in the formed elements and not in the plasma or serum. Even the cellular elements exhibit well marked and characteristic differences in their conduct toward oxidase and peroxidase reagents. Furthermore, various observers do not seem to be agreed with regard to the oxidizing properties shown by the formed elements of the blood. Thus it was shown by Klebs in 1868 (250) that pus has the power of bluing guaiacum directly. On the other hand, according to Linossier (273), if one uses a freshly prepared tincture of guaiacum the superficial portion of the resin having been previously removed by washing with alcohol, the blue color is not produced upon addition of pus, but only after the addition of hydrogen peroxide. Hence, according to Linossier, pus (or white blood corpuscles) contains a peroxidase, but no oxidase. More recently, however, Meyer (300) has shown that in the perfectly fresh state the thick leucocyte layer obtained by centrifugalizing the blood of patients suffering with myelogenous leucemia, gives no reaction with guaiacum. If, however, water be added and the mixture shaken a few moments, a very intense blue color develops. On the other hand, if the corpuscles be shaken with serum or with an isotonic salt solution no blue color develops. According to Meyer this shows that the leucocytes contain an oxidizing ferment (oxidase) which first becomes liberated or activated by the solution of the cells in water. Similarly a few drops of leucemic blood diluted with such a large amount of water that scarcely any blood color was visible gave with guaiacum a deep blue color. He also observed that fresh untreated pus gives no guaiacum reaction, but that when shaken with distilled water it gives the guaiacum reaction at once. He also found that these aqueous extracts of pus and leucocytes from myelogenous leucemia could oxidize phenolphthalin. He concludes, therefore, that the polynuclear and probably also the mononuclear, neutrophile cells contain a substance extractable with water which blues guaiacum without the addition of hydrogen peroxide, or in other words, that it contains an intracellular oxidizing ferment killed by boiling, but not at 73° C., and which, as found for other oxidases by Bach and Chodat, contains an oxygenase and a peroxidase.

Reference has already been made to the fact that A. and L. Lumière and Chevrotier (81) have prepared a protoplasmic extract of blood corpuscles which exhibits the properties of an oxidase to a marked degree, as shown by its power to oxidize guaiacum, guaiacol, hydroquinon, etc.

In the light of these facts there can scarcely be any doubt that certain of the leucocytes contain both oxidases (see also Portier (331, 332,)) and peroxidases.

Dr. Norman Roberts, working in the Hygienic Laboratory on the peroxidase activity of the urine in health and disease, has examined the urines of 175 diseased persons and also the urines of a considerable number of normal individuals for peroxidases. As nearly as he has been able to discover, the peroxidase reaction of urine is not specifically characteristic of any of the diseases studied, except such as involve an active inflammation of the genito-urinary tract. It does seem to be constant, however, for active inflammations of this character and is due to the leucocytes and possible in some instances to certain epithelial cells which these urines contain. He has also shown that in fresh blood smears and fresh sections of highly vascular tissues the majority of the leucocytes are stained blue on treatment with the peroxidase reagent (a solution containing cresol, para-phenylene diamin, and hydrogen peroxide), whereas the red cells of the blood and the cells characteristic of the tissues examined remained unstained. (See also Winkler (453).) As is well known, pus also actively decomposes hydrogen peroxide.

In the light of these facts there can scarcely be any doubt that certain of the leucocytes contain oxidases, peroxidases, and catalases.

We have seen further that Senter (400) has prepared a very active catalase from defibrinated blood, free from hemoglobin and from any oxidase or peroxidase. While, therefore, the blood undoubtedly contains oxidases, peroxidases, and catalases, it has gradually come to be recognized that its oxygen-carrying power can not be due entirely to oxidases and peroxidases, for the reason that this oxygen-carrying power persists after boiling, and after treatment with acids and alkalis. Indeed it seems to persist as long as the blood pigments are not deprived of their iron. Thus according to Moitessier (305) the so-called peroxidase reaction of the blood, upon which is based most of the chemical tests for blood, is not really due to a peroxidase but to hemoglobin and hematin. According to this author the non-ferruginous blood pigments, such as hematoporphyrin, do not exhibit such reactions. Czyhlarz and von Fürth (131) have also arrived at the conclusion that the so-called peroxidase reaction of the blood is due to hematin and not to a peroxidase. Lesser (267) also obtained the guaiacum reaction with blood which had been boiled. He also is of the opinion that the reaction is due to the blood pigment and that the iron-free derivatives of hemoglobin do not give it. Whitney (450) also concludes that it is the iron of the hemoglobin and its iron-containing derivatives which are responsible for the guaiacum test and similar reactions. Buckmaster (104, 105) also regards the oxidizing power of blood in the presence of hydrogen peroxide as due to a pseudo-peroxidase and in some way dependent on the part played by iron in the hemoglobin molecule, although the precise way in which the iron acts is still obscure. Studies on the oxygen-carrying power of blood toward solutions containing hydrogen peroxide, phenolphthalin, and alkali have also been made by Kastle and Amoss (241) and later by Kastle (240), who has recently recommended this reaction as a chemical test for blood. It has been shown that the amount of phenolphthalin oxidized both in the presence and absence of hydrogen peroxide is proportional to the quantity of hemoglobin present, and that while the oxygen-carrying power of the blood is somewhat diminished, it is by no means entirely destroyed even after repeated boiling. The formed elements of the blood therefore contain oxidases, peroxidases, catalases, and a more stable oxygen-carrier, viz, hemoglobin, or, in certain of the lower animals, hemocyanin. To the catalases we owe the active decomposition of hydrogen peroxide into water and molecular oxygen, as shown by blood and pus. To the oxidases we owe the oxidation of guaiacum, phenolphthalin, and similar substances, as shown by aqueous solutions of the leucocytes. To the peroxidases we owe those oxidations which take place only in the presence of hydrogen peroxide or a similar compound, as also shown by the leucocytes. These properties are all lost on boiling. On the other hand, certain oxidations by means of hydrogen peroxide are induced by hemoglobin and its iron-containing derivatives, and hence we find that the blood can still induce certain oxidations by hydrogen peroxide even after it has been boiled. The fact that these carriers become inactive with the splitting off of the iron which they contain is a matter of great interest in view of the part played by iron and manganese in the activation of the oxidases and in view of the relation of iron to active nucleo-proteids.

The fact that hemoglobin and its iron-containing derivatives oxidize various chromogenic substances in the presence of hydrogen peroxide has been turned to practical account in hematological and forensic investigations. This subject has recently been discussed at length by Kastle (240) in a communication entitled "Chemical Tests for Blood," in which there is given a reasonably complete bibliography of the extensive literature of this subject.

IRON, COPPER, AND MANGANESE IN THEIR RELATION TO THE OXIDIZING FERMENTS. ARTIFICIAL OXIDASES, PEROXIDASE ACCELERATORS, AND AUXILIARY OXYGEN CARRIERS.

According to Spitzer (407) the oxidizing power of animal tissues is referable to certain nucleo-proteids which they contain, and the oxidizing power of these substances is in turn referable to combined iron. In this connection it is of interest to note that so far as is known the most stable and most perfect oxygen catalysts occurring in the animal organism contain either copper or iron. According to Wells (verbal communication) the blood of certain marine forms has been found to contain zinc. The fact that hemoglobin and its iron-containing derivatives are all capable of inducing the oxidation of such substances as guaiacum, phenolphthalin, etc., by means of hydrogen peroxide or other compounds of this nature, whereas the iron-free blood pigments can not accomplish such oxidations, speaks strongly in favor of the assumption that the activity of such oxygen carriers is dependent on iron in organic combination. (*See* Kastle (240)), "Chemical Tests for Blood," Bulletin No. 51, Hygienic Laboratory.) In this connection it is also of interest to note that Floyd (171) found nearly twice as much ash in the skin of the negro as in that of whites, and nearly twice as much iron in the ash of the negro's skin as in the ash of whites. According to this author the pigment appears to originate in the outer layer of the true skin, and in all probability is the product of the alteration of the red coloring matter of the blood. It has also been shown experimentally that certain iron salts accelerate biological oxidations. Thus Battelli and Stern (36) have found that the quantity of carbon dioxide produced by the action of an extract of the muscle of the horse or dog on calcium lactate is considerably increased by the presence of small amounts of ferrous sulfate.

It has been shown by Bertrand (56, 57, 58) that the oxygen-carrying power of laccase is in some way associated with the presence of manganese. By fractional precipitation with alcohol he was able to resolve a certain specimen of laccase into two portions, one of which was poorer and the other richer in manganese than the original sample. The oxidizing powers of these three specimens were proportional to their manganese content, as may be seen from the following table:

No. of specimen.	Quantity of manganese in ash.	Oxygen absorbed in 1½ hours by 50 c. c. of a 2 per cent solution of hydroquinon in the presence of 0.2 gram of the specimens.
	Per cent.	*c. c.*
1	0.159	19.1
2	.126	15.5
3	.098	10.6

He also succeeded in showing that the laccase contained in lucerne is poor in manganese and comparatively inactive toward hydroquinon. In the presence of a small amount of manganese (1 milligram in the form of the sulfate) the oxidizing power of lucerne laccase is greatly increased. That such is the case is evident from the quantities of oxygen absorbed by a solution of hydroquinon in the presence of manganese alone, lucerne laccase alone, and a mixture of manganese and laccase, thus:

	Oxygen absorbed.
	c. c.
1. Manganese alone	0.3
2. Lucerne laccase alone	.2
3. Laccase + manganese	6.3

In other words, the effect of the manganese on laccase poor in this constituent was to increase the oxygen absorption twenty-five times. Other metals, such as iron, aluminium, cerium, zinc, copper, calcium, magnesium, and potassium, were found to be incapable of increasing the oxidizing power of laccase.

He therefore arrived at the conclusion that the oxidizing ferments as we ordinarily recognize them consist in reality of two portions, one organic and very unstable with which we associate those properties usually characteristic of the ferments as a class, and a second portion, which might be called the co-ferment, mineral or organic in its nature, and which with the first substance forms the really active system. Bertrand (59) therefore is disposed to regard manganese as the co-ferment of laccase, just as calcium is the co-ferment of pectase, and hydrochloric acid the co-ferment of pepsin.

In a later communication Bertrand (56, 57) proved that various manganese salts can bring about the oxidation of various oxidizable substances, such as hydroquinon, etc., in the air, and that the quantity of oxygen absorbed varies with the nature of the manganese salt employed, being greater with the manganese salts of the organic acids. The several amounts of oxygen absorbed by hydroquinon in the presence of various manganese salts are shown in the following table:

Salt of manganese.	Oxygen absorbed.	Salt of manganese.	Oxygen absorbed.
	c. c.		c. c.
Nitrate	1.5	Acetate	15.7
Sulfate	1.6	Salicylate	16.3
Chloride	1.8	Lactate	17.6
Formate	7.4	Gluconate	21.6
Benzoate	15.3	Succinate	22.1

It would seem, therefore, that those manganese salts which are most easily hydrolyzable are the most efficient oxygen carriers. According to Bertrand, therefore, when a manganese salt finds itself in contact with water it hydrolyzes in the sense of the equation—

$$R''\mathrm{Mn} + \mathrm{H_2O} = R''\mathrm{H_2} + \mathrm{MnO}.$$

As is well known, however, manganous oxide is spontaneously oxidizable, and in the course of this oxidation molecular oxygen is split into two atoms, one of which combines with the manganous oxide to form the peroxide, the other going to oxidize the hydroquinon or other oxidizable substance, which ordinary molecular oxygen is powerless to oxidize. Thus

$$MnO + O_2 = MnO_2 + O,$$

and

$$C_6H_4(OH)_2 + O = C_6H_4O_2 + H_2O.$$

In the presence of an acid, $R''H_2$, and a substance like hydroquinon the latter is oxidized. Thus a further amount of hydroquinon is oxidized and the manganese salt regenerated, thus:

$$R''H_2 + MnO_2 = R''Mn + H_2O + O,$$

and

$$C_6H_4(OH)_2 + O = C_6H_4O_2 + H_2O.$$

In harmony with this view Bertrand proved that while manganese peroxide is stable in dilute acids it is reduced on adding hydroquinon, with the production of quinon and a manganous salt. He was therefore led to consider the oxidases whose oxidizing powers are so greatly intensified by a manganese salt as special combinations of manganese and an acid radicle, the latter probably of a proteid nature and partaking of the nature of a ferment and having just the affinity necessary to maintain the metal in solution. According to this conception the manganese would be the really active element of the oxidase, so far as the activation and transfer of oxygen is concerned, whereas the acid albuminoid radicle would impart to the ferment its other properties, such as its conduct toward heat, solubility, etc. According to Rey-Pailhade (343) the reducing ferment which he has described under the name of "philothion" possesses the properties of the acid albuminoid radicle of the oxidases.

Other observers have found manganese in oxidases. Thus Lepinois (264) found both manganese and iron in the oxidase of the roots and leaves of aconite and belladonna. Carles (110) found manganese in quantity in the oxidase of valerian root. According to Vitali (440) the oxidase of pus contains a trace of manganese, and to this it owes its oxidizing properties. Aso (15) likewise found both manganese and iron in the ash of the oxidase of tea leaves. Portier (332) has also shown that the activity of the oxidase of the branchies of *Artemis exoleta* is generally increased by the addition of small amounts of sulfate of manganese.

According to Bach and Chodat (31) manganese is also the active agent of the peroxidases. Kelley (249) has recently shown that pineapple plants containing a high percentage of manganese contain a more active oxidase than plants which have only small percentages of this element.

On the other hand, oxidases have been obtained which contain no manganese. Thus Sarthou (357-359) found the oxidase of *Schinus molle* to contain iron, calcium, and sodium, but no manganese; according to this author the oxidizing power of this oxidase is proportional to its iron content. Vadam (138, 139) found iron but no manganese in the ash of an oxidase from hellebore. According to Stoecklin (112), manganese is not present in the ash of the oxidase from horse-radish.

It is evident, therefore, that certain metals, such as iron, manganese, and copper, one or more of them, enter into the composition of the oxygen carriers present in the living organism as essential ingredients. In this connection it is interesting to note that small amounts of manganese greatly accelerate a number of chemical processes. Thus according to Villiers (141) if one boils equal volumes of a saturated solution of oxalic acid and hydrochloric acid containing 25 per cent of HCl, and nitric acid equally dilute, there is not produced any disengagement of gas, even after a long time; on the other hand, if one adds to the solution a trace of a manganese salt the action develops instantly and there is produced a regular disengagement of carbon dioxide and nitrogen. This author has compared the action of a manganese salt to that of a mineral ferment (*ferments mineraux*). In another communication he (142) describes the use of small amounts of a manganese salt in the destruction of organic matter in toxicological analysis. Gigon and Rosenberg (143) have observed that the presence of small amounts of manganous or ferrous sulfate increases very markedly the digestion of starch by the diastatic blood ferment and by the pancreatic diastase.

Attempts have been made, therefore, to prepare artificial oxidases by means of certain manganese and iron compounds, and some observers have pointed out various analogies existing between certain metallic salts and the oxidases. Thus according to Trillat (136) when a small quantity of manganese chloride is added to a dilute solution of fresh egg albumin (3 parts to 100 of water) and the solution made faintly alkaline with caustic soda or potash there is produced a solution which in the raw state rapidly turns brown in the air, the change of color beginning on the upper surface of the liquid. In contact with the air this solution has been found to blue guaiacum and to oxidize hydroquinone with the production of quinone, and pyrogallic acid to purpurogallin. Thus with a solution of alkali alone a given quantity of pyrogallic acid gave 0.102 gram of purpurogallin, whereas under the same conditions the albuminous solution containing manganese gave 0.617 gram of purpurogallin. It was observed, further, that the oxidation of the phenols is accompanied by the evolution of carbon dioxide. With certain colloids the artificial oxidase resulting from admixture with the manganese salt could be precipitated with alcohol and the precipitate redissolved with water without losing its oxidizing powers. For the most part heat destroys the oxidizing power of such solutions. Thus when heated to 105° C. for twenty minutes the solution no longer oxidizes guaiacum nor certain diphenylmethane derivatives. The effect of heat is also seen in the several amounts of oxygen absorbed by pyrogallic acid under the influence of the fresh and heated solutions; thus:

	Absorption in cubic centimeters.		
1. With the fresh unboiled solution the following amounts of oxygen were absorbed	45.0	38.0	47.0
2. With the boiled solution	0.0	5.0	3.0

It is evident, therefore, that colloidal solutions of manganese hydroxide obtained by the action of caustic soda on manganese salts in the presence of albumin and similar colloidal substances exhibit properties closely resembling those of the natural oxidases.

Garrigou (180) has found certain metals to exist in a colloidal condition in mineral waters. According to this author, therefore, such substances possess the properties of natural oxidases, and to them he ascribes the beneficial results which attend the drinking of such waters and which are the result of more vigorous oxidations in the tissues. A. and L. Lumiére and Chevrotier (280) have confirmed the work of Trillat (436) on the oxidizing power of colloidal solutions of manganese and the general analogy of such solutions to the oxidases. According to these authors, colloidal solutions of any metal capable of existing in two or more states of oxidation should exhibit properties similar to those shown by the oxidases, the state of division of the colloidal substance also determining the oxidizing power of the substance. As a matter of fact, they found that emulsions of iron and cerium in albumin, gelatin, or gum, have the power to blue guaiacum, oxidize hydroquinon, pyrogallol, guaiacol, and para-phenylene diamin, but less actively than emulsions of manganese. These authors also sought to utilize these properties in destroying the bacterial poisons, since it is known that such poisons are destroyed by powerful oxidizing agents. As a matter of fact, they found that the death of an animal by tetanus toxin was greatly delayed by the administration of these artificial oxidases, and that with a slightly acid oxidase and tetanus toxin the animal did not show the slightest sign of intoxication.

Robin and Bordet (350) have also investigated the action of artificial oxidases in infectious diseases and have arrived at the conclusion that the colloidal metals, especially manganese, promote oxidation in the tissues and intensify metabolism.[a] They claim also to have confirmed the work of A. and L. Lumiére and Chevrotier (supra) on the curative action of such substances. Fouard (174) has observed that the halides of the alkalis and the alkaline earths exercise a catalytic action in the fixation of oxygen by the polyphenols, and that the chlorides of the rare earths act even more vigorously as oxygen carriers (175). In the title of the latter article, (175), he refers to these reactions as of the oxidase type, and yet there is nothing to indicate that the activity of these carriers is destroyed by heat, so that, to my mind, the analogy is only the product of a very active imagination.

Martinand (288) has also pointed out that the oxides of the alkalis and alkaline earths which form soluble peroxides and percarbonates, give reactions similar to organic peroxidases. He calls attention to certain analogies existing between the oxides of the alkalis and the alkaline earths and the organic oxidases on the one hand and to those existing between certain metallic salts and the peroxidases on the other. He is of the opinion that these inorganic oxidases can be considered as similar to the organic oxidases in that the former are made up of a peroxidase, which is a salt of the metal, and an oxygenase, which is the peroxide formed by the action of the air on the salt. Like the organic oxygenases, the inorganic oxygenase can be replaced by hydrogen peroxide. These analogies fall short, however, in that they fail to take into account the destructive action of heat on the oxidases and peroxidases. Wolff (155) has shown that certain mineral salts can play the part of peroxidases. Thus, if a trace of ferrous sulfate (less than one part per million) be added to an old tincture of guaiacum, or to a fresh tincture containing a trace of hydrogen peroxide, a very intense blue coloration is obtained. On heating the solution containing ferrous sulfate and hydrogen peroxide, it loses its power to blue guaiacum, and traces of mineral acids prevent the bluing of guaiacum by the ferrous sulfate and hydrogen peroxide just as they prevent the action of the peroxidases, the dosage required to inhibit the reaction being the same in both cases. According to this author these reactions closely resemble those produced with plant extracts containing peroxidase, such as extract of malt, etc., and he is of the opinion that special interest attaches to these observations in view of the fact that salts of iron, like the peroxidases, are very widely distributed among living things. He has shown (156) further that certain colloidal compounds of iron are practically identical with the peroxydiastases (a name recently proposed by

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[a] In this connection see also See ([illegible]) and Schade ([illegible]).

Bertrand for the peroxidases). Thus with infinitesimal amounts of colloidal ferrocyanide of iron he claims to have been able to reproduce all of the reactions of the peroxidases. Thus it is filterable without loss of activity, its activity is weakened after one minute's boiling, and traces of mineral acids reduce its activity. It is also sensitive to an excess of hydrogen peroxide, as has been observed by Bach and Chodat (27) for the plant peroxidases. Other compounds of iron and cyanogen exhibit similar properties, although less active than the ferrocyanide. In another communication, entitled "Artificial Peroxydiastases," Wolff (457) points out that ferrous ferrocyanide acts in all respects like a natural enzyme. On the other hand, Wolff and Stoecklin (460) have found that while colloidal ferrous ferrocyanide acts like a peroxidase toward phenols, it fails to accelerate the oxidation of hydriodic acid by hydrogen peroxide. They conclude, therefore, that the catalytic action of vegetable peroxidases, such as extract of malt, on this reaction, is due to a special enzyme which may be eliminated by careful purification of the peroxidases. Continuing these investigations, Wolff (458) has shown that a feebly alkaline solution of ferrous ferrocyanide acts as an oxidase toward hydroquinone. He also points out that the alkalinity of the liquid plays a principal rôle in such oxidation phenomena. Thus the manganous salts employed by Bertrand (56, 57, 58) in his studies of the effect of manganous salts on the oxidation of hydroquinon, were always found to be alkaline toward alizarin sulfate, helianthin, and even in certain instances to turmerol. It was also shown by Wolff that the activity of neutral manganous salts, such as the sulfate is considerably increased by the addition of traces of pyridin, which does not precipitate manganese from its solutions.

Euler and Bolin (168) have also studied the oxidation of hydroquinone in alkaline solution under the influence of manganese salts. The relationship of the manganous salt and alkali is compared to that of the enzyme and co-enzyme. These authors have also shown that laccase has no action on hydroquinone in the absence of manganous salts, thereby confirming the work of Bertrand (57, 58). They showed further that laccase is not of the enzyme type, since solutions of it could be boiled for three minutes without destroying their activity. It was also found that salts of the hydroxy-acids, such as Rochelle salts, sodium citrate, calcium glucinate, and sodium mucate, accelerate the oxidation of hydroquinone in a marked manner when manganese salts are present, and these authors have suggested that laccase owes its activity to the presence of such salts.

According to Stoecklin (413), iron tannate can act as a peroxidase, causing the oxidation of guaiacol and tyrosin and the conversion of alcohol into aldehyde by hydrogen peroxide.

During the last two or three years Dony-Henault (143) and his co-workers have carried out a number of investigations on the subject of the oxidases. According to this author the oxidizing action of laccase is not really due to a specific enzyme but to the presence of manganese salts, the activity of which is stimulated by hydroxyl ions. He is inclined to the opinion that too much stress has been laid on the inhibition of catalytic processes by heat, as indicative of the action of enzymes. On the other hand he is of the opinion that in the presence of colloids, manganese salts would be decomposed in aqueous solution by heating to 100° C., and thereby lose their oxidizing power. Artificial laccase has been prepared by this author (144) by adding iron formate to blood serum and twice precipitating with alcohol. The substance thus obtained was found to show the reactions characteristic of natural laccase, but much less intensely. Still other artificial laccases were prepared by precipitating gum arabic with alcohol in the presence of manganous and other salts. A very active preparation was obtained by precipitating a solution containing 10 grams of gum arabic, 1 gram of manganese formate, and 0.4 gram of crystallized sodium carbonate in 50 c. c. of water, with alcohol. The precipitate was filtered off, redissolved in water, filtered, and reprecipitated. This second precipitate when washed and dried constituted the active, artificial laccase. The fact that artificial laccases can be obtained without resorting to the use of albuminous substances in these preparations, as was done by Trillat (136), indicates that Bertrand's idea that laccase contains an acid proteid radical is incorrect. Dony-Henault is of the opinion, therefore, that laccase as a distinct oxidizing enzyme does not exist. He explains the hardening and blackening of the juice of the lac-tree as due to the action of manganese in the presence of alkalis.

This author has also criticised all previous work on the oxidases on the ground that whereas in the study of the digestive ferments we have employed for the investigation the true substratum upon which they normally act, thus for diastase we use starch and for invertase, cane sugar, the action of the oxidases has been studied upon artificial or fictitious substrata, such as guaiacol, hydroquinone, etc. He therefore distinguishes between diastatic or enzymic action and catalytic action, the former being specific whereas the latter is variable or general, and he concludes that for the reason that the known oxidizing ferments act upon a number of oxidizable substances, they can not be true enzymes. He is of the opinion, therefore, that the belief in the existence of oxidases proper does not rest upon a sufficiently rigorous experimental basis. On the other hand it would seem preposterous to assume the existence of several dozen or a hundred distinct lipolytic ferments in the liver for the reason that an aqueous extract of this organ can hydrolyze a large number of ethereal salts, and it would indeed be surprising to find that an oxidase, such as laccase, could oxidize but one substance, viz, laccol, and not other phenols, especially in view of the close analogy existing between this compound and the phenols as to constitution and properties. On the other hand certain of the oxidases are apparently as specific in their effects as any other ferment; thus tyrosinase has the power of bringing about the oxidation of tyrosin, whereas the latter is not acted upon by laccase. Effront (156) is of the opinion that the individuality of tyrosinase and laccase has been established definitely, so that conclusions of this kind are open to considerable objection.

Sjolleman (403) states that by adding a solution of Seignette salt to a solution of manganous sulfate or acetate, and then hydrogen peroxide and then sodium hydroxide, one obtains a dark brown solution of manganous oxide, which shows itself to be colloidal on dialysis and toward electrolytes, and which retains its dark brown color for several weeks. This solution has been found to give characteristic reactions for the oxidases, and to decompose hydrogen peroxide like potassium permanganate. The author calls attention to the occurrence of manganese in plants as of interest in this connection, and states that manganese probably plays an important rôle in the oxidations occurring in the soil. (*See* Schreiner and Reed (390).

PEROXIDASE ACCELERATORS AND AUXILIARY OXYGEN CARRIERS.

In his investigation of the ozonic reactions of certain mushrooms it was shown by Schoenbein (378) that guaiacum blue (believed by him to be an ozonid), which results from the action of the juices of such fungi on tincture of guaiacum, can in turn oxidize other oxidizable substances, as a result of which action the guaiacum blue is reduced and decolorized. It has been recently shown by Kastle (239) that peroxidase oxidations also are in many cases greatly accelerated by the action of certain phenols. Thus it was observed by Kastle and Porch (246) that the oxidation of guaiacum, paraphenylen diamin, and phenolphthalin by hydrogen peroxide under the influence of the peroxidase of milk is greatly accelerated by phenol, the cresols, and by beta-naphthol, and similar results have been obtained by Kastle (239) with peroxidases from a number of different sources. According to this author the peroxidase accelerators probably act in the capacity of auxiliary oxygen carriers and are themselves more or less completely oxidized in such processes. Thus if *P* represents the peroxidase, *A* the auxiliary oxygen carrier, and *B* the peroxidase reagent, that is, the substances whose oxidation is really under observation, the changes taking place might be represented graphically as follows:

(1) $$P + H_2O_2 \rightarrow PO_2;$$

and

(2) $$PO_2 + A = P + AO_2;$$

or

(3) $$2PO_2 + A = 2PO + AO_2,$$

and

(4) $$AO_2 + B = AO + BO,$$

and since, as has been shown by Bach and Chodat (27) the oxidases consist of an oxygenase (an organic peroxide) and a peroxidase, it is readily conceivable that oxidations accomplished by the oxidases might also be accelerated by certain oxidizable substances. Thus laccol itself might function as a peroxidase accelerator and in its oxidation by laccase it might accomplish the oxidation of other less readily oxidizable substances contained in the juice of the lac-tree.

CATALASES.

One of the most characteristic properties of hydrogen peroxide is the ease with which it is decomposed into water and oxygen. Thus in the earlier researches on this substance by Thénard (420, 421) it was observed that this decomposition can be effected by finely divided metals, by the oxides and peroxides of the heavy metals, and also by substances of animal origin such as fibrin. We have already seen that these observations were greatly extended by Schoenbein (383). He proved that certain plant juices blued guaiacum directly; after standing for a short time, however, they lose this property but still retain the power to blue guaiacum in the presence of hydrogen peroxide, although this property is also lost after a time. Such extracts and juices were also found to have the power of decomposing hydrogen peroxide with the evolution of oxygen gas, and as a general thing he observed that extracts and tissues having the power to decompose hydrogen peroxide have also the power to blue guaiacum if hydrogen peroxide is present. Yeast and rennin, however, proved to be exceptional in their conduct in this regard. Both of these substances were found by Schoenbein to decompose hydrogen peroxide, yet neither had the power to blue guaiacum in the presence of hydrogen peroxide. According to Schoenbein the power to decompose hydrogen peroxide and to blue guaiacum containing small amounts of the peroxide were not specific properties of any particular class of substances but rather general properties of all soluble ferments, since the soluble ferments exhibit these changes and since the loss of its specific activity on the part of any particular soluble ferment by the action of heat or otherwise is attended with the loss of its activities toward hydrogen peroxide and a mixture of this substance with guaiacum. As a matter of fact, he was led to regard the decomposition of hydrogen peroxide into water and oxygen as accomplished by these various agents as the prototype of all fermentation (^{383}; page 335). These erroneous ideas prevailed for a number of years. Thus, according to Flügge (^{172}) cited by Loew (^{278}), all enzymes can decompose hydrogen peroxide, and in 1900 Babcock and Russell (^{17}) attempted to measure the activity of galactase, the proteolytic ferment of milk, by the activity of an aqueous extract of the separator slime toward paraphenylen-diamin and hydrogen peroxide (von Storch's reagent).

Gradually, however, facts accumulated in the literature tending to show that the power to decompose hydrogen peroxide and to blue guaiacum by means thereof were not general properties of all soluble ferments, nor were the two properties necessarily correlated, but that they were specific properties of distinct sets of substances. Thus Bergengrün (^{45}) in 1888 observed that the fibrin ferment does not decompose hydrogen peroxide. Similarly Jacobson (^{223}) demonstrated that the property of certain soluble ferments to decompose hydrogen peroxide could be destroyed by heating to certain temperatures and by the action of acids and alkalis without in any way injuring the specific activity of the particular ferment. So also Raudnitz (^{338}) from his studies on the oxidases of milk reached the conclusion that the substance in milk which decomposes hydrogen peroxide is essentially different from that which gives the guaiacum reaction. Lepinois (^{265}) also pointed out as the result of his studies on the ferments decomposing hydrogen peroxide, that there is not always a parallelism between the quantities of oxygen liberated and the intensity of other reactions, such as the bluing of guaiacum, the reddening of guaiacol, etc. Finally, in the course of the examination of a number of samples of tobacco for oxidases, Loew (^{278}) observed that aqueous extracts of certain of the samples of cured tobacco gave an abundant evolution of oxygen on the addition of hydrogen peroxide, without giving a blue color with guaiacum. He proved the absence of diastase and emulsin in these samples of tobacco, so that evidently the power of such extracts to actively decompose hydrogen peroxide is not due to either of these ferments. He showed further that active preparations of certain of the ferments, such as diastase, emulsin, and papayotin, from other sources, are without action on the peroxide, so that evidently the power to decompose hydrogen peroxide is not a general property of enzymes. He found further that certain liquids and extracts have the power of bluing guaiacum in the presence of hydrogen peroxide, which have not the slightest power of decomposing the peroxide into water and oxygen, and conversely, certain kinds of animal and vegetable matters have the power of vigorously decomposing hydrogen peroxide without giving the peroxidase reaction with guaiacum. Thus it often happens that samples of cured tobacco have the power of decomposing hydrogen peroxide, ofttimes very actively, and yet have no power to blue guaiacum either alone or in the presence of hydrogen peroxide. On the other hand, an aqueous extract of the fresh green leaves of tobacco, containing one-fifth of its volume of absolute alcohol, showed both the oxidase and peroxidase reactions with guaiacum, whereas such extracts had no power to decompose hydrogen peroxide. It would seem, therefore, that the power to decompose hydrogen peroxide and the oxidase and peroxidase reactions ordinarily exhibited by plant and animal tissues and secretions are distinctly different properties. According to Loew (278), therefore, the decomposition of hydrogen peroxide by plant and animal tissues is due to a special enzyme, to which he gave the name "catalase."[a]

Still other observations point to the specific nature of catalase. Thus in 1902, Pozzi-Escot (333) observed that catalase did not give the guaiacum reaction nor oxidize hydroquinon. Somewhat later

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[a] The name "catalase" (spelled by these authors "katalase"), together with the names "oxygenase" and "peroxidase," has recently been objected to by Moore and Whitley ([illegible]) as being ill chosen. According to these authors "there is no reliable evidence of this destruction of hydrogen peroxide being due to an enzyme at all." They point out that it is not specific, and that the decomposition can be accomplished by every ferment solution of whatever type, by nearly all animal and vegetable fluids, and by means of numberless inorganic catalysts. "In any case it is absurd," according to these authors, "to give it a name which belongs to or includes the whole vast range of catalytic actions." Every true enzyme is a catalase in the sense that it acts catalytically, and why a catalytic agent which happens to act upon hydrogen peroxide and which furthermore has never been shown to be a specific enzyme should be dignified by the name "catalase" is difficult to conceive. Every investigator in this field will no doubt appreciate the force of some of these objections. At the same time there are doubtless many who would be inclined to question the exclusion of catalase from the group of soluble ferments. The mere fact that innumerable substances decompose hydrogen peroxide has nothing to do with the question. One might as well object to our looking upon invertase as an enzyme for the reason that innumerable acids can hydrolyze cane sugar, and while many names in science are ofttimes nothing more than apt and striking catchwords, they have taken such a firm hold in the minds of those dealing with the subject that it is practically impossible to replace them. An instance of this kind is met with in the term "catalysis" itself, which in its original meaning simply begs the question as to the inherent causes of all phenomena of this kind. To say that an agent simply acts by its presence is merely emphasizing the most obvious phase of the whole phenomenon, and conveys no insight into the causes thereof, which after all are the things which we are chiefly concerned in discovering, and yet it is quite likely, from the present drift of things, that the term "catalysis" will remain long after contemporary chemists have ceased to have a voice in the shaping of chemical thought. If a committee of chemists, philologists, and advocates of the simple spelling were all to get together with the express purpose of introducing new names for the oxidases, peroxidases, and catalases it is more than likely that the result would be something far less euphonious and suggestive and of less real meaning than the names which these substances now bear.

Senter (400) isolated from the blood a catalase of remarkable activity, which he called "hemase," and which was without action upon guaiacum even in the presence of hydrogen peroxide. More recently L. Liebermann (269) has shown that an aqueous extract of the mesenteric fat of the hog and rabbit decomposes hydrogen peroxide energetically, without being able to oxidize guaiacum. According to Neumann-Wender (449) yeast, by heating to 45° to 50° C., loses its power to induce alcoholic fermentation, whereas the yeast catalase is only destroyed at 68° to 70° C. He also showed that yeast in which the maltase has been destroyed still retained its catalytic power. L. and P. Liebermann (270) have also shown that the catalase of malt is destroyed by heating extract of malt to 80° C., or by shaking it with mercuric oxide and magnesia, whereas the solution can still exhibit the guaiacum reaction, especially if oil of turpentine be added. Hence they conclude that only the peroxidase is concerned in the oxidation of the guaiacum. Pure hemoglobin is powerless to effect the decomposition of hydrogen peroxide, and yet it acts as a powerful oxygen carrier in a mixture of hydrogen peroxide and guaiacum. The general drift of modern opinion seems to be, therefore, that the catalases and peroxidases are distinctly different ferments.

So far as its occurrence is concerned, catalase seems to be one of the most widely distributed of any of the enzymes. Thus from his own observations Loew (278) arrived at the conclusion that there does not exist a group of organisms, or any organ, or even a single vegetable or animal cell, that does not contain some catalase.

According to Loew (278), vegetable catalase exists in two forms, one insoluble and the other soluble in water. These he designated "α-" and "β-catalase," respectively. According to him, α-catalase is probably a compound of β-catalase with a nucleo-proteid, while the β-catalase is an albumose, and can be liberated from the insoluble form (α-catalase) by means of a dilute alkali. He found that cured tobacco contains but little soluble catalase (β-catalase), whereas well-sweated tobacco contains a great deal. Water alone dissolves only traces of α-catalase, while the prolonged action of water at moderately high temperatures gradually splits off β-catalase, especially if a small amount of sodium carbonate be added. Whether the vegetable catalases obtained from different sources are identical in composition and nature, and whether they are identical with the animal catalases, is as yet unknown. By most observers the catalases are regarded as enzymes. They may be obtained in perfectly clear solution; thus Senter (400) found that the catalase of blood passes through the Berkefeld filter. They are destroyed by heat; Loew (278) found that vegetable catalase is destroyed at 80° C.; according to Senter (400), hemase is destroyed at 65° C.; Neumann Wender (449) found yeast catalase to lose its activity at 70° C., although the dry yeast catalase could be heated to 100° C. without being destroyed.

The kinetics of the decomposition of hydrogen peroxide by catalase (hemase) and various inorganic catalysts, especially the colloidal metals (inorganic ferments), has been exhaustively studied by Senter (400) and also by Bredig (96, 97). The decomposition of hydrogen peroxide both by catalase (hemase) and by the colloidal metals has been shown to conform to the law of an irreversible monomolecular process. With constant quantities of the catalyst the velocity of the decomposition has been found to be directly porportional to the concentration of the peroxide within certain limits. At small concentrations, however, both with the ferment and inorganic catalysts, the decomposition is proportionately slightly greater than with greater concentrations. In other words, the hydrogen peroxide itself, or some impurity which it contains, slightly inhibits the decomposition. With constant amounts of the hydrogen peroxide, especially at small concentrations, the decomposition has been found to be proportional to the quantities of catalase (hemase) or inorganic catalyst present. With more concentrated solutions of the catalase, however, both with the hemase and colloidal metal, the velocity of the reaction increases more rapidly than the increase in the concentration of the catalyst. A rise of temperature of 10° C. increases the velocity of the decomposition of hydrogen peroxide by platinum 1.7, and by hemase 1.5, whereas, according to van't Hoff (216) for most chemical processes the velocity is doubled by such an increase in temperature.

The power to decompose hydrogen peroxide as shown both by hemase and the inorganic catalysts is greatly inhibited by certain poisons and foreign substances, such as hydrocyanic acid, hydrogen sulfide, hydroxylamin, metallic nitrates, etc., at very great dilutions; and in this connection Loevenhart and Kastle (275) made the interesting observation that the activity of the catalase of hog liver was greatly inhibited by ammonium sulfocyanide, whereas it was rendered even more active by thiourea.

Since the recognition of catalase as a specific enzyme its occurrence and distribution in various animal and vegetable tissues has been investigated by a number of observers. We have seen that Spitzer (407) measured the catalytic power of various animal tissues toward hydrogen peroxide with the view of determining their relative oxidizing power. The various animal tissues have been found to vary greatly in catalytic power. Thus, according to Battelli and Stern (35) the liver contains the most and the brain the least amount of catalase of any of the tissues examined. They found, further, that the tissues of the guinea pig contain more catalase than those of the frog. Similarly Jolles and Oppenheim ([illegible]) found that the tissues of warm-blooded animals have a greater catalytic activity than those of cold-blooded animals, such as the fish. In 1899 Carrière (112) examined a number of normal and pathological secretions for indirect oxidases (peroxidases and catalases). He obtained no evidence of catalase in the urine of 10 normal persons, nor in the urines of persons suffering from diabetes, hysteria, or chlorosis. On the other hand, in nephritis (Bright's disease), tuberculosis, and in acute inflammation of the lungs, the urine was found to decompose hydrogen peroxide. With other normal and pathological liquids his results are not sufficiently numerous to permit of any general conclusions.

The catalytic power of the blood toward hydrogen peroxide in normal and diseased conditions has also been investigated by Jolles (227, 228) and later by Jolles and Oppenheim (229). According to these observers the catalase of blood is contained exclusively in the formed elements, and is roughly proportional to the hemoglobin. They have determined the quantity of hydrogen peroxide in grams which is decomposed by 1 cubic centimeter of blood in normal and diseased conditions; this they have designated as the "catalase value." For normal blood this was found to be 23, whereas for blood in certain diseased conditions the catalase value was considerably less than for normal blood; thus in tuberculosis it ranged from 10 to 13, and in nephritis from 8 to 13, the most interesting and remarkable decrease occurring in carcinomatous conditions, in which condition the catalase values of the blood fall to 1.3 to 2.1. The blood of the two sexes and venous and arterial blood exhibited no differences in catalytic power.

Still more recently Winternitz and Meloy (454) have studied the occurrence of catalase in the human blood and tissues and its variation in certain diseases. These authors have reached the conclusion that there is no marked variation in the catalytic activity of human tissues due to the age of the individual. In this same connection, Mendel and Leavenworth (298) observed that catalase does not seem to be less abundant in very young embryos than in the adult. Winternitz and Meloy (supra) found the catalytic activity of the tissues to vary greatly in certain diseased conditions; thus in nephritis the activity fell off, especially in the kidney itself. In two cases of eclampsia the catalytic activity of the blood was not reduced. In pneumonia, the lung, in the stage of red hepatization, was found to have an increased catalytic activity, due in all probability to the increased number of red blood cells. In tuberculosis a decrease in the catalytic power of the various tissues was observed, but there was no reduction in diabetes mellitus nor in jaundice. The tissues in one case of congenital syphilis showed a marked lowering in catalytic activity, as did also the blood in one case of asphyxiation by illuminating gas.

THE FUNCTION AND NATURE OF CATALASE.

Very little is known regarding the function of catalase or of its mode of action on hydrogen peroxide. We have seen that according to Schoenbein's theory of oxygen-activation the inactive oxygen of the air is decomposed during processes of autoxidation, the negatively polarized atom uniting with molecular oxygen to form ozone and the positively polarized atom combining with water to form hydrogen peroxide (an antozonide), and, further, that through the action of certain carriers, such as finely divided platinum and the red coloring matter of the blood and what are now known as the peroxidases, the relatively inactive oxygen of the hydrogen peroxide is ozonized. This ozonized product can then accomplish the oxidation of easily oxidizable substances, such as guaiacum, etc., or it can react with another portion of the hydrogen peroxide, forming water and molecular oxygen after the manner of ozone itself, or of an ozonide, such as lead peroxide. In other words, the catalysis of hydrogen peroxide was of the nature of a secondary process in the sequence of changes occurring in the formation of ozone and ozonides as the result of the autoxidation of easily oxidizable substances. The occurrence of catalase in the organic world is, according to Loew, too general and widespread to be accidental, and hence the enzyme must have a certain significance. In considering its possible significance he pointed out that hydrogen peroxide results as either a primary or secondary product in the autoxidation of many readily oxidizable organic substances. Hence he regarded it as conceivable at least, that hydrogen peroxide might also be produced in the living cell as the result of the respiratory process. The accumulation of such a substance as hydrogen peroxide would undoubtedly prove harmful to the life of the organism, and hence he conceived the function of catalase to be to destroy the hydrogen peroxide as fast as formed. The ferment would thus afford an important protection against the accumulation of this poisonous substance, and the oxygen thus liberated could be again utilized for a continuance of the respiratory process. He also advanced the idea that just as catalase loosens the affinities of oxygen in hydrogen peroxide, so also it might loosen the affinities in certain other compounds, as the result of which they might be more easily decomposed or oxidized by the protoplasm.

Herlitzka ([illegible]) has found that within certain limits the greater the concentration of catalase the greater the concentration of peroxidase required to produce oxidation. This points to a protective action on the part of catalase against the peroxides of the organism. Shaffer ([illegible]) is also of the opinion that through the decomposition of hydrogen peroxide by catalase into water and molecular (inactive) oxygen, the tissues are protected against injurious oxidation. In like manner Battelli and Stern (37) claim to have shown that catalase inhibits to some extent the oxidations produced by ferrous sulfate in the presence of animal tissues. Hence they, too, are of the opinion that the function of catalase may be to prevent the excessive oxidation of organic substances in the living cell. According to Jolles and Oppenheim (227, 229) the catalases of the blood eliminate oxygen from oxyhemoglobin, and this oxygen is then transferred to the tissues by the oxidases.

On the other hand the fact that catalase can not decompose either the substituted organic peroxides, such as ethyl hydroperoxide, or the oxygenases (Bach and Chodat,[29]), both of which are probably more powerful oxidizing agents than hydrogen peroxide, is difficult to reconcile with the view that the function of catalase is to protect the organism against excessive oxidation. As pointed out by Chodat (116), the only property of the catalases of which we have any certain knowledge at present is their power to decompose hydrogen peroxide into water and molecular oxygen. In this respect they differ from all other known catalysts. Thus all other catalysts of this kind can not only decompose hydrogen peroxide but they also have the power of rendering active the oxygen thereof. Thus finely divided platinum actively decomposes hydrogen peroxide. It also gives the guaiacum reaction both with hydrogen peroxide and with molecular oxygen. As shown by Kastle and Clarke (242) potassium iodide actively decomposes hydrogen peroxide at 100° C.; it was also found to greatly accelerate the oxidation of formic acid by hydrogen peroxide at 60° C., whereas potassium chloride neither actively decomposes hydrogen peroxide nor does it increase its oxidizing powers. If, therefore, the catalases are really exceptional in this regard, they certainly afford a very remarkable class of exceptions, and of such a nature, indeed, as to be altogether inexplicable at the present time. On the other hand the thought naturally suggests itself that they are not exceptional at all, but that they simply represent special cases under the general rule governing the conduct of such substances toward hydrogen peroxide. Thus it is readily conceivable that the catalases, like the peroxidases, combine with hydrogen peroxide to form an unstable holoxide derivative, thus:

$$K + H_2O_2 = H_2KO_2.$$

This might prove to be so unstable, however, that it would decompose in the sense of the equation—

$$H_2KO_2 = K + H_2O + O,$$

before it would have a chance to effect the oxidation of any oxidizable substance at hand; or in the event that oxidations occurred, it is conceivable that the catalase itself, K, might be more readily oxidizable than guaiacum or any of the peroxidase reagents, in which event we would have—

$$H_2KO_2 = H_2O + KO.$$

This would explain the fact that while powerful catalysts, the catalases are not unlimited in their power to effect the decomposition of hydrogen peroxide. In this connection it is of interest to note that Senter (408) found blood-catalase (hemase) to be oxidized at all temperatures above 0° C. It is easier, however, and more in harmony with what we know regarding the conduct of other catalysts, to suppose that both of these changes would occur simultaneously, viz.,

$$H_2KO_2 = K + H_2O + O,$$

and

$$H_2KO_2 + \text{Guaiacum} = K + H_2O + \text{Guaiacum-blue},$$

in which event the given substances would exhibit the properties of both a catalase and a peroxidase; and it may be after all, that when examined more closely, the catalases will show peroxidase reactions. As it is, the two sets of substances, if they are really distinct, are certainly found in the closest and most intimate association in both plant and animal tissues.

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LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

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The following *bulletins* [Bulls, Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

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No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

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Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis*; by Ch. Wardell Stiles.

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*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN No. 60
April, 1910

A STUDY OF THE ANATOMY OF WATSONIUS (n. g.) WATSONI OF MAN

AND OF

NINETEEN ALLIED SPECIES OF MAMMALIAN TREMATODE WORMS OF THE SUPERFAMILY PARAMPHISTOMOIDEA

By

CH. WARDELL STILES
and
JOSEPH GOLDBERGER

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A STUDY OF THE ANATOMY OF WATSONIUS (n. g.) WATSONI OF MAN,

AND OF NINETEEN ALLIED SPECIES OF MAMMALIAN TREMATODE WORMS OF THE SUPERFAMILY PARAMPHISTOMOIDEA.[a]

By Ch. Wardell Stiles,
Professor of Zoology,
and Joseph Goldberger,
Passed Assistant Surgeon, United States Public Health and Marine-Hospital Service.

SUMMARY.

The present paper contains the results of an anatomical study of one parasite of man (*Watsonius watsoni*) and comparison with 19 other closely related trematodes, part of which were sent to us for determination.

Watsonius watsoni, originally classified as *Amphistoma*, later as *Cladorchis*, represents the type of a new genus. Its testes are one caudad of the other, instead of side by side, as heretofore interpreted.

The families *Paramphistomidæ* and *Fasciolidæ* should be raised to superfamilies as *Paramphistomoidea* and *Fascioloidea*. *Paramphistomoidea* contains three families, *Gastrothylacidæ*, *Paramphistomidæ*, and *Gastrodiscidæ*.

These families may be divided into various subfamilies, genera, and subgenera, as shown in the table of contents (pp. 5–6) and in the various keys (pp. 15, 16, 50, 61, 62, 63, 74, 131, 173, 247, 249.

Anatomically, the group in question is very interesting, but a careful study of any given species is exceedingly tedious because of the thickness of the specimens. The projection method was found to be the most satisfactory in preparing drawings to illustrate the topography.

Of rather special interest is the perisuctorial cavity, which may be very large in some species. Dorsal and ventral mesenterium-like bands traverse this cavity, binding the oral sucker to the body parenchyma. The structure in question is strongly suggestive of a rudimentary body cavity, the absence of which is characteristic for the group of Flat Worms, to which these parasites belong.

We have been fortunate in having for study some of Cobbold's original material of *Amphistoma stanleyii* and *A. collinsii*, with the result that we accept *Pseudodiscus* as a valid genus. Anatomically, the fact is important that these species show a very complicated structure of the oral sucker and its pouches, the latter being separated from the former by intermediate bulbs.

Summaries of the separate groups may be found in the respective diagnoses and keys.

---

[a] Submitted for publication September 23, 1909.

INTRODUCTION.

Among the parasites recently sent to this laboratory for determination have been several species of amphistomes, which prove to be new to science. In studying them it has been found necessary to compare certain known forms especially the so-called *Cladorchis watsoni* of man. Fortunately for this work, we have had at our disposal some of Cobbold's original material of species of the little-known genus *Pseudodiscus* and one series of sections of the original material of *Cladorchis watsoni.*

The study has resulted in certain changes in classification and the proposition of several new genera and subgenera.

TAUTONYMY IN GENOTYPES.

One of us (Stiles) has upon several former occasions expressed the view that in the case of genotypes absolute or virtual tautonymy is exceedingly desirable. In accordance with this view we have, whenever the occasion presented itself, purposely formed the names so that either virtual or absolute tautonymy results. A consistent application of this principle would do much to reduce confusion in classification.

BIBLIOGRAPHIC REFERENCES.

Bibliographic references and systematic names of parasites refer to the citations given in Stiles & Hassall's Index Catalogue of Medical and Veterinary Zoology (Authors, Bull. 39, United States Bureau of Animal Industry; Trematoda, Bull. 37, Hygienic Laboratory, United States Public Health and Marine-Hospital Service).

TERMINOLOGY.

As some of the technical terms used in this paper are not in common use in helminthology, it may be well to give a few words of explanation in regard to them.

Oral sucker and pharynx.—The initial suctorial organ of the digestive tract of trematodes is usually named the "oral sucker," while the term "pharynx" is reserved for an organ of less constant nature which develops in the esophagus. In recent amphistome literature the term "pharynx" has been substituted by authors for the "oral sucker" of the amphistomes. We agree with Looss that this substitution does not appear to be well founded and we therefore revert to the term "oral sucker."

Acetabulum. The term "acetabulum" is reserved exclusively for the "caudal sucker," homologous with the "ventral sucker (acetabulum)" of the distomes.

Evaginations of oral sucker.—This is a general term used to designate any kind of an evagination from the lumen of the oral sucker; the evagination may be paired or circular.

Bulbs and pouches.—As these terms are used in this bulletin, a "pouch" is a cecal evagination, regardless of its histological structure or position relative to the sucker. In some species (as in *Pseudodiscus*) the pouch is separated from the sucker by a "bulb" namely, not a cecal structure.

The pouches vary somewhat in histological structure in different species. The walls usually present a loose mesh or parenchyma-like texture with few if any muscular elements; in some cases the meshwork appears more or less condensed, approaching the structure of the oral sucker as the latter occurs in *Homalogaster philippinensis* and *Watsonius watsoni*.

The bulbs agree with the oral sucker histologically, whether the latter be dense or loose in structure.

Testes and testicles.—We favor the use of the term "testis" and "testes" for the two male glands of the trematodes; if these glands are divided, as in *Pleorchis* or as in the cestodes, the subdivisions may well be called "testicles."

Genital pore. The genital pore is the opening which leads from the external (ventro-median) surface of the worm into the genital atrium.

Genital atrium. This is the cavity or depression into which the genital papilla projects. Its dimensions vary greatly in the different forms in which it occurs. In some it is partly divided into a ventral and a dorsal chamber by a more or less sharply developed projecting ring.

Porus hermaphroditicus.—The external opening of the ductus hermaphroditicus is the hermaphroditic pore and usually discharges into a genital atrium.

Topographic terms.—In a paper now in course of preparation, one of us (Stiles) is proposing a departure in the topographic terminology of the trematodes, and some of the terms are used in this bulletin. In brief, longitudinal and transverse straight lines are drawn at the periphery of the various organs; the longitudinal lines bound fields, the transverse lines bound zones. Portions of the body bounded by other than straight lines (as that portion bounded by the intestinal ceca) are termed "areas." Organs are then located with reference to these fields, zones, and areas. Thus, the testicular zones may coincide, overlap, abut, or be separate; the testicular fields may coincide, overlap, abut, or be separate. An ovary may be described as in the pretesticular, testicular, or posttesticular zone, or in the extratesticular, testicular, or intertesticular field; a given organ may be in the prebifurcal zone, preacetabular zone, postacetabular, postovarial, postuterine zone, etc. The body is also divided into five transverse zones, each representing 20 per cent of the body length; these zones, beginning at the oral pole, are called the first, second, third, fourth, and fifth. It is believed that by aid of this system, descriptions may be made more exact than they frequently are at present, and that, especially in the case of tabular keys, the system will be found useful. A key to the figured species of distomes is now being formed on this principle; a preliminary study, based upon about 150 illustrations, has thus far been found to be very satisfactory.

SUPERGENERIC SYSTEMATIC UNITS.

During recent years, especially since 1898, the tendency in helminthology has been to raise species to generic rank, and genera to subfamily or even to family rank. In this tendency, helminthology has simply followed in the wake of other specialties in zoology. If in continuing this tendency we fail to recognize certain other systematic units, such as the superfamily (ending in *oidea*), and tribes and subtibes (ending in *idi* and *ini*), the danger is present that natural groups will be separated, units coordinate in rank will not be coordinate in actual value, and the classification will become confused.

It is true that the superfamily and the tribes and subtribes have not yet been recognized in the International Code, but they have been used by so many authors that they can be looked upon as recognized units. We here recognize the superfamily as standing between the suborder and the family, and we adopt for it the ending (*oidea*) proposed by Gill. The tribe (ending *idi*) and the subtribe (ending *ini*) we recognize as standing between the subfamily and the genus.

It would seem to us wise to raise the former trematode families *Fasciolidæ* and *Paramphistomidæ* to superfamily rank *Fascioloidea* and *Paramphistomoidea*, in order to leave room for expansion of systematic units made advisable because of recent changes in taxonomic conceptions in these groups. The present paper deals entirely with the *Paramphistomoidea*.

TECHNIQUE.

The material which forms the basis of this paper consisted, in the main, either of specimens sent to us for determination or of preserved specimens forming a part of the helminthological collection of the United States National Museum. Some of this latter material was quite old, dating back as far as 1875 (*Ps. stanleyii*), but was remarkably well preserved. In the beginning of this work, after studying the external characters of the individuals of a species, we made "toto" mounts of stained and cleared specimens and a series of transverse and, if sufficient material was available, of sagittal and frontal sections. The general topography of the internal characters could more or less clearly be made out from the "toto" mounts and this would be corrected or amplified by a study of the serial sections. This method of procedure is obviously only applicable where several individuals, at least two, of one form are available for the study of the internal anatomy. Inasmuch as some of our most valuable material consisted of forms comprising not more than two individuals and only one of these was available for such study, the following method of procedure was devised by one of us (Goldberger) and applied with exceedingly satisfactory results. The external characters of the specimen selected were first carefully noted and then such drawings made as seemed desirable. These always included one of the ventral and another of the profile aspect, the outlines being made with the aid of the camera lucida. The specimen was next stained. We have used both carmine (para-carmine, carmalum, acetic acid alum-carmine) with and without counterstain (picric acid) and Mayer's hæmalum, but on the whole the carmine stains without counter stain gave us the most satisfactory pictures. After staining we dehydrated with alcohol and cleared in xylol. The cleared specimen was now examined under the microscope and drawings and notes made of such points in the internal topography as could be made out. After this the specimen was infiltrated and embedded in paraffin of a melting point of 54° C., careful note being taken of the orientation; this was always such as to give transverse sections at right angles to the median sagittal plane of the worm. Depending on the size of the worm the sections were cut at either 10μ or 20μ. In this connection it may perhaps be mentioned that, as has been repeatedly observed by one of us (Goldberger), the most favorable temperature for obtaining good ribbons of sections of this thickness is between 27° and 29° C.

The next step is the reconstruction of the worm from measurements of the sections with the ocular micrometer. The reconstruction is by projection on paper of a series of significant points at selected levels in either a ventral or a sagittal view. The base line in the former case may be one of the lateral margins, or, more simply and equally satisfactorily, the median sagittal plane (represented as the median longitudinal axis); in the case of the sagittal projection the profile line of the dorsum is used as the base line. The projected points belonging to the various organs are connected by lines which give, in effect, the outline, on a suitable scale, of the projected shadow of the body of the worm and of the organs or systems of organs. A convenient scale is 50 for sections of 20μ and 100 for those of 10μ in thickness. On this scale each section is represented in projection as 1 mm. thick, and consequently the frontal apparent length of the worm would be represented as equal to the total number of the sections in millimeters. Given a satisfactory series of sections, this method enables one to work out the topography of the organs or systems of organs with a high degree of precision. It must be said, however, that it is also very tedious and time consuming. Our figures 1, 2, 23, 24, 45, 46, 61, 62, 72, 73, 83, 84, 94, 95, 105, 106, 139, 140, 192, and 193 were constructed in this way.

PARAMPHISTOMOIDEA, new superfamily.

SUPERFAMILY DIAGNOSIS.—*Trematoda, Malacocotylea, Digenea:* Acetabulum caudoterminal, subterminal, or ventral close to caudal end. Oral sucker and esophagus present, ceca 2. Hermaphroditic. Genital pore ventro-median, preequatorial, pretesticular, preovarial.

Male organs: Testes 1 or 2, usually preovarial.

Female organs: Vitellaria paired.

TYPE FAMILY.—*Paramphistomidæ.*

This superfamily is practically *Paramphistomidæ* Fischœder. It should, we believe, be divided into three families, as follows:

KEY TO FAMILIES OF PARAMPHISTOMOIDEA.

*A*¹. Body discoidal; divided into an anterior (cephalic) and a posterior (caudal) portion; venter with many, large papillæ; acetabulum ventral, at caudal end; ventral pouch absent . . . . *Gastrodiscidæ*, p. 249.

*A*². Body not discoidal, not divided, venter not provided with many large papillæ:
  *B*¹. Ventral pouch present . . . . *Gastrothylacidæ*, p. 15.
  *B*². Ventral pouch absent . . . . *Paramphistomidæ*, p. 60.

GASTROTHYLACIDÆ, new family.

FAMILY DIAGNOSIS.—*Paramphistomoidea* (p. 15): Ventral pouch present.

TYPE GENUS.—*Gastrothylax* Poirier, 1883.

This group has heretofore been considered a genus in the family *Paramphistomidæ*, subfamily *Paramphistominæ*, but the presence of a ventral pouch separates it so radically from the other forms that distinct family rank seems justified. The typical, and thus far the only, subfamily *Gastrothylacinæ*, contains 13 species, which may be divided at present into 4 more or less natural groups. At least one of these groups (type *gregarius*) will doubtless soon require further subdivision. The question as to whether these groups should at present be given generic or subgeneric rank is one upon which there may be a very legitimate difference of opinion, but we believe that the entire tendency of the present day, wise or unwise as it may eventually prove to be, is to consider the differences in question as generic, and in the present paper we shall so regard them.

GASTROTHYLACINÆ, new subfamily.

SUBFAMILY DIAGNOSIS.—*Gastrothylacidæ* (p. 15): Body elongate, venter straight to concave, dorsum convex, cephalic end attenuate, rather pointed, straight, may curve slightly dorsad, caudal end slightly attenuate to rounded, usually slightly constricted immediately preacetabular, in transverse section more or less circular but form influenced by pouch. Ventral pouch opens slightly postoral, extending nearly or quite to acetabulum. Acetabulum relatively small, terminal to ventro-subterminal, slightly sunken, margin not raised. Genital pore without sucker. Excretory pore postvesicular or nearly so, in acetabular zone, caudad of pore of Laurer's canal. Oral sucker without evagination; esophagus without muscular thickening; ceca narrow, wavy or not wavy, long or short, end postequatorial.

Male organs: Testes 2, considerably smaller than acetabulum, coarsely lobate, rarely postovarial, chiefly preovarial, postequatorial, not widely separated from acetabulum; musculosa never enormous; cirrus-pouch absent.

Female organs: Ovary and shell-gland in testicular or posttesticular zone, never pretesticular; vitellaria extend from oral sucker to acetabulum, nearer ventral pouch than body wall, more ventral than dorsal, follicles in small groups; Laurer's canal anatomically prevesicular, but because of curve may lie partly in vesicular zone; uterus of 2 types (see genera).

Eggs: Operculated (at least in some forms).

TYPE GENUS.—*Gastrothylax* Poirier, 1883.

The four genera here recognized for the subfamily *Gastrothylacinæ* may be distinguished by the following key:

KEY TO GENERA OF GASTROTHYLACIDÆ, GASTROTHYLACINÆ.

A^1. Vas deferens and cephalic half of uterus in separate, right and left, largely extrasuctorial fields; uterus crosses to other side near equator of body; testicular fields separate, zones coincide; type *crumenifer*....................*Gastrothylax*, p. 16.

A^2. Vas deferens and cephalic half of uterus chiefly or entirely in suctorial field:

  B^1. Testicular fields separate (lateral), zones coincide:

    C^1. Vesicula seminalis with a straight and a coiled portion; testes in inter, extra, and cecal areas; type *wellmani*....................*Wellmanius*,[a] p. 51.

    C^2. Vesicula seminalis without straight portion; testes inter or postcecal; type *gregarius*....................*Carmyerius*, p. 50.

  B^2. Testicular fields coincide or overlap (median), zones coincide or overlap; type *elongatus*....................*Fischœderius*, p. 17.

GASTROTHYLAX Poirier, 1883.

GENERIC DIAGNOSIS.—*Gastrothylacinæ* (p. 16): Vas deferens and cephalic half of uterus in separate right and left, largely extrasuctorial fields, uterus crosses to other side near equator of body. Testicular fields separate, zones coincide and, chiefly, postcecal, ovary in intertesticular field. Cross section of ventral pouch triangular, with apex dorsad, base ventrad. Ceca end preovarian, preacetabular, in fourth zone, not wavy.

TYPE.—*G. crumenifer* (Creplin, 1847).

This genus is apparently Asiatic, and at present contains two species, which can be easily distinguished by the following key:

Ceca end pretesticular, body 9 to 11 mm. long; type host *Bos indicus*. [Probably from Asia]....................*G. compressus* Brandes, 1898.

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[a] In general we prefer masculine endings in zoological generic names.

Ceca end in testicular zone; body 9 to 18 mm. long; type host *Bos indicus*, Asia; also in *Bos kerabau*, Ceylon..*G. crumenifer* (Creplin, 1847).

FISCHŒDERIUS, new genus.

GENERIC DIAGNOSIS.—*Gastrothylacinæ* (p. 16); Vas deferens and cephalic half of uterus chiefly or entirely in suctorial field. Testicular fields median, coincide or overlap, zones coincide or overlap, one testis more dorsal than the other, inter or postcecal; vesicula without straight portion. Ventral pouch divides body into 3 longitudinal body segments, a dorsal segment containing uterus, and 2 ventro-lateral segments.

TYPE SPECIES.—*F. fischœderi*. Asiatic.

This genus, which we dedicate to Fischœder (the well-known author who has done so much to advance our knowledge of the mammalian amphistomes), contains four very closely allied species, which may be distinguished by the following key:

a^1 Ceca end posttesticular, postovarian, in acetabular and fourth zone; testicular fields overlap, zones nearly coincide; body 8 to 10 mm. long; type host *Palonia frontalis*, Java..*F. cobboldii* (Poirier, 1883).
a^2 Ceca end pretesticular, preovarian, preacetabular:
  b^1 Genital pore on vertex of prominent hemispherical bulging; testicular fields and zones overlap:
    c^1 Ceca end in third zone; ovary and shell gland not intertesticular; body 10 to 20 mm. long; type host "*Palonia*" *frontalis*, Java; also in *Bos kerabau* in China, and *Anoa depressicornis*..............................*F. elongatus* (Poirier, 1883).
    c^2 Ceca end in fourth zone; ovary and shell gland intertesticular; body 6.4 mm. long; type host *Bos kerabau*, Ceylon..............................*F. fischœderi*, p. 17.
  b^2 Genital pore not on vertex of prominent hemispherical bulging; ceca end in third zone:
    c^3 Testicular zones overlap; ventral pouch not continued posttesticular; body 6.6 to 15. 5 mm. long; type host *Bos* sp., Siam......................*F. siamensis*, p. 28.
    c^4 Testicular zones coincide; ventral pouch continued posttesticular; body 6 to 7 mm. long; type host *Bos kerabau*, probably from Asiatic region. *F. ceylonensis*, p. 39.

FISCHŒDERIUS FISCHŒDERI, new species.

[Figs. 1 to 10.]

SPECIFIC DIAGNOSIS.—*Fischœderius* (p. 17): Body 6.4 mm. long, 2 mm. broad, 2 mm. thick; buff color (alcohol specimen); rather conical, greatest transverse diameter near equator, greatest dorso-ventral diameter in caudal third; attenuated cephalad, and very slightly caudad; longitudinal axis somewhat curved, concavity ventrad; cephalic extremity bluntly pointed; caudal extremity blunt; dorsum convex longitudinally; venter slightly concave longitudinally; transverse sections nearly circular, but tending toward a bluntly rounded triangle, with apex ventrad, especially in equatorial region. Surface smooth except for a few blunt papillæ in cephalic region and on lip of aperture of ventral pouch. Opening of ventral pouch 0.5 millimeter from cephalic margin; pouch begins with a narrow neck, which at the genital pore widens into a large cavity, extending dorsally to near genital glands, ventrally slightly farther; transverse section of aperture and neck crescentic, of the cavity rather triangular with apex ventrad. Genital pore seemingly very slightly postbifurcal on a prominent bulging. Acetabulum 1.04 mm. in diameter, terminal, slightly sunken below surface of body, with 0.5 mm. aperture directed caudad but very slightly ventrad. Mouth terminal; oral sucker 0.6 mm. long, slightly larger than esophagus; perisuctorial cavity roomy;

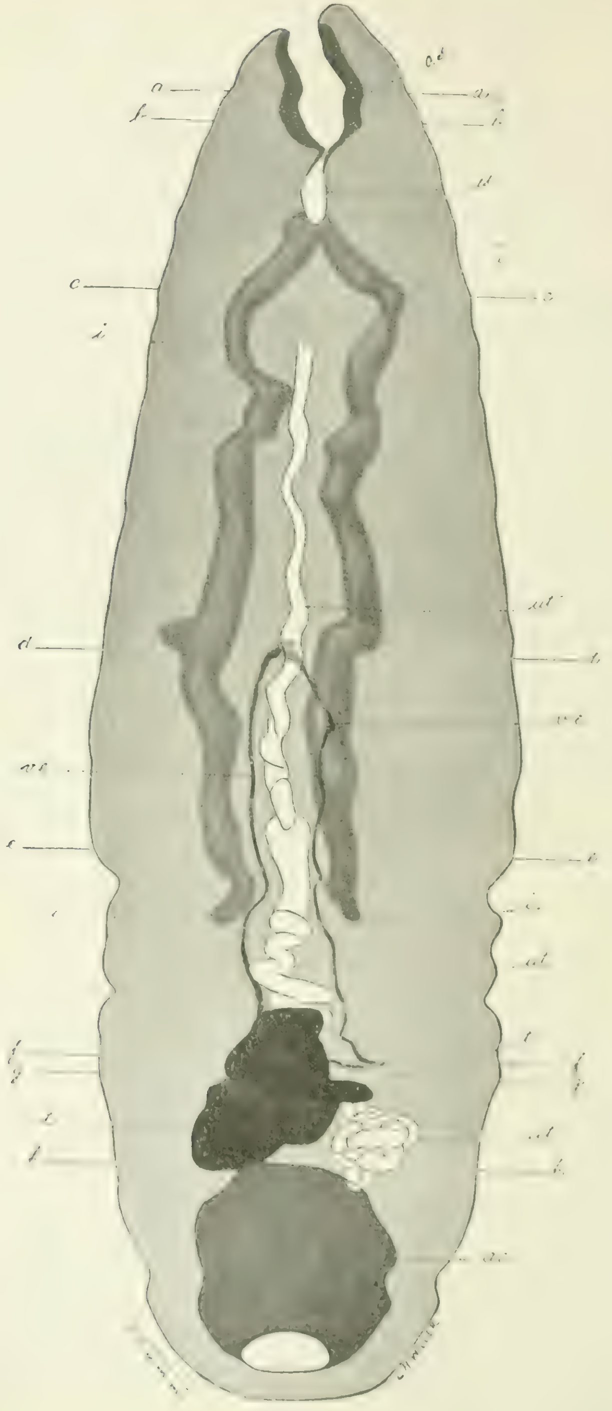
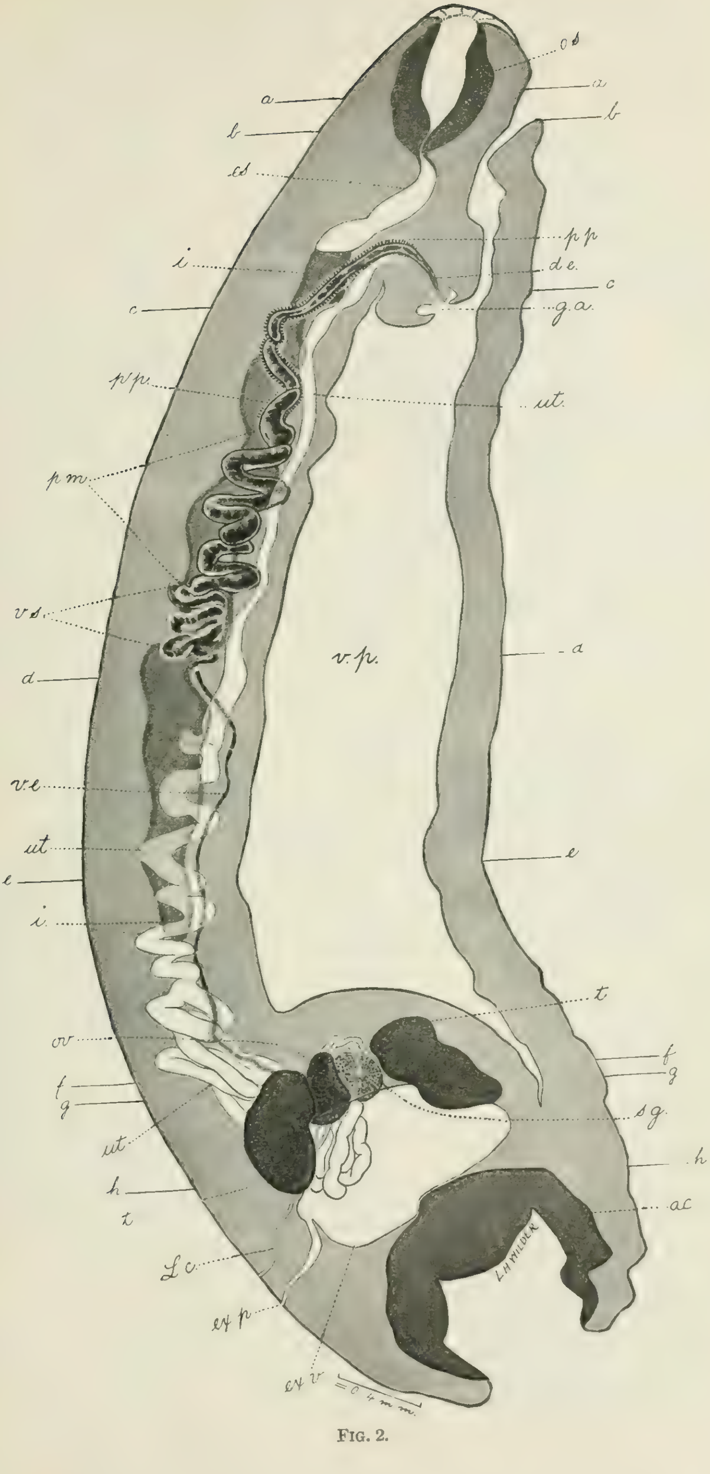


FIG. 1.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= 0.4 mm.
L.H. WILDER

FIG. 2.

intestinal bifurcation about one-sixth of body length from oral margin; intestinal ceca extend to caudal end of equatorial third of body. Excretory pore 0.16 mm. caudad of pore of Laurer's canal; excretory canal runs ventro-cephalad to dilated vesicle cephalad of acetabulum.

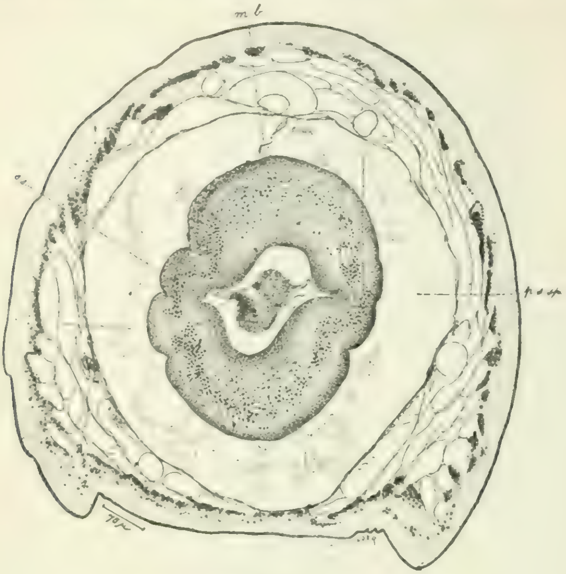


FIG. 3.

Male organs: Testes somewhat lobate, one ventral of the other, separated by ovary, one extending to right the other to left of median line, thus zones and fields overlap; the testes lie between acetabulum and fundus of ventral pouch; vasa efferentia arise on dorsal aspect, unite slightly cephalad of equator of body; vas deferens much coiled; pars prostatica less coiled; ductus ejaculatorius probably discharges independently of metraterm.

Female organs: Ovary and shell gland between testes; vitellogene glands consist of sparsely scattered follicles in ventrolateral body segments extending from slightly caudad of apparent genital pore to acetabulum; uterus extends from shell gland caudad, dorsally, turns cephalad, and runs between vasa efferentia, dips ventrally, extending cephalad, ventrally of vas deferens, to genital pore; Laurer's canal runs from oviduct dorso-caudad, opens on plane of cephalic margin of acetabulum, about 0.16 mm. cephalad of excretory pore.

Eggs: Few in number, 135 by 75μ in sections.

TYPE.—U.S.B.A.I. 15328.

HABITAT.—In (? organ of) *Bos kerabau*, from Ceylon.

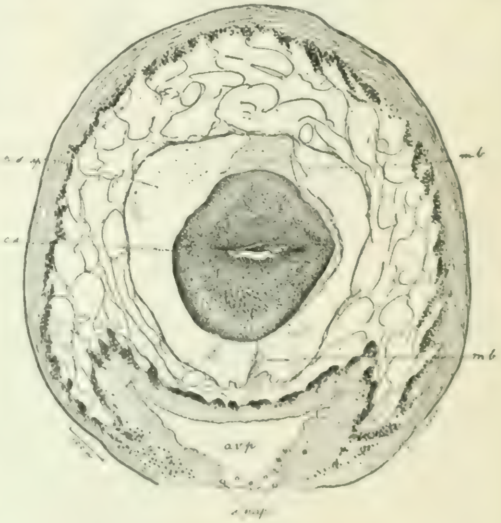


FIG. 4.

SOURCE OF MATERIAL.—The single specimen studied was taken from bottle numbered 3377, containing a label with the following legend:

Name, *Gastrothylax elongatus* Poir. Host, *Bos kerabau*. Locality, Königsberg, Tiergarten (from Ceylon). Determined by Fischœder. Collected by Fischœder. Presented by Luehe, June, 1902.

The specimen was sectioned with a view to serving for purposes of comparison, but was found to differ from *G. elongatus* in several respects. Its new number is 15328.

EXTERNAL CHARACTERS.

Size.—The measurements taken from the sectioned specimen give a length of 6.4 mm. and greatest transverse and dorso-ventral diameters of about 2 mm. each.

Color.—The specimen was of a buff color.

Form.—In form it closely resembled *F. elongatus*, being somewhat spindle or cone shaped. While the greatest transverse diameter was in the equatorial region, the greatest dorso-ventral diameter was in the caudal third of the body. This appears to have been brought about by slight compression or shrinking from side to side of the caudal portion. The longitudinal axis is curved with the concavity ventrad. The cephalic extremity is bluntly pointed; the caudal is broad though somewhat attenuated as compared with the equatorial region. In the cephalic portion the transverse section is nearly circular but with some flattening ventrally in the region above the aperture of the ventral pouch (fig. 3); in the equatorial region the form in transverse section tends to assume a rounded-triangular outline (figs. 6 and 7) with the apex of the triangle ventrad.

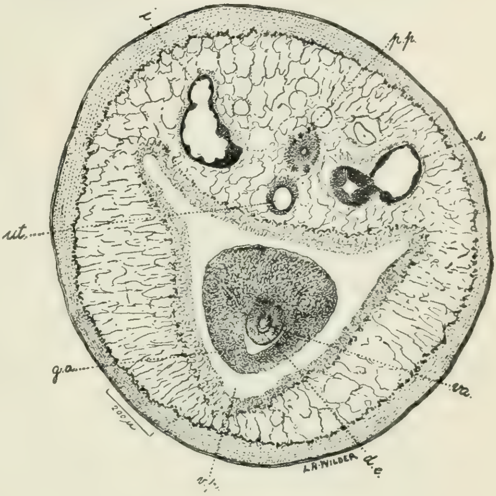


Fig. 5.

Surface.—The surface cuticle is unarmed; but a few blunt papillæ were observed on the cephalic portion and on the lip of the aperture of the ventral pouch (fig. 4).

Ventral pouch.—About 0.5 mm. caudad of the cephalic margin there is a transverse slit which marks the ventral aspect of the animal and serves as the aperture of the ventral pouch (see fig. 4). The lip of the aperture curves latero-cephalad from the median line forming two lateral ridges (see fig. 3), which rapidly fade out. Included between these ridges is a portion of the ventral aspect of the cephalic extremity which is flattened and continuous with the dorsal wall of the neck of the pouch. The aperture gives entrance to a ventro-dorsally narrow passage which extends caudad to the level of the genital pore, beyond which this neck dilates rather rapidly to form the body of the pouch (fig. 2). The pouch extends caudad to a point slightly cephalad of the genital organs. The fundus of the pouch bulges somewhat cephalo-ventrad into the lumen in such a manner that the dorsal wall of the pouch is the shortest, the ventral the longest, and the lateral walls intermediate between the two. A dorso-ventrally very narrow, crescentic, slit-like prolongation of the pouch extends caudad in front of the ventral testis to the plane of the caudal aspect of the latter (figs. 2, 8, 9). In transverse section the aperture of the ventral pouch is a crescentic slit, measuring 0.45 mm. from horn to horn and about 0.1 mm. ventro-dorsally; the canal above (cephalad of) the genital pore retains the crescentic outline of the aperture, but below this level it tends to a triangular form, which becomes well defined in the body of the pouch. The apex of this triangle, like that of transverse sections of the body of the animal, is directed ventrad. Corresponding to the position of the angles of this triangle the inclosing body is constricted, marking off three segments, a dorsal and a right and left ventro-lateral, of which the dorsal is somewhat the largest (see figs. 6 and 7). In the equatorial region the longitudinal axis of the pouch and that of the body are substantially identical.

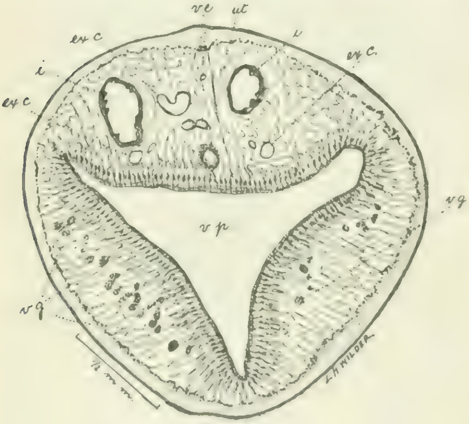


FIG. 6.

Genital pore.—On the dorsal wall of the neck of the ventral pouch at the vertex of a large muscular somewhat hemispherical bulging is the genital pore (figs. 2, 5). The pore leads into an atrium, the dorsal wall of which is in the form of a papilla, at the vertex of which is the opening of the ductus ejaculatorius and beneath and adjacent to this that of the metraterm. In the only specimen available for study the genital pore seemed to be in a plane slightly post-bifurcal. This appears to be due, however, to an artificial crowding or bending caudad of the hemispherical bulging which bears it. It is easily conceivable that a crowding or bending in the opposite direction would bring the pore to, or close to, the aperture of the ventral pouch.

Acetabulum.—This muscular organ, measuring about 1.04 mm. in diameter, occupies the caudal terminal portion of the body. It is, like that of *F. elongatus*, dome shaped, having an aperture measuring 0.05 mm., which is directed caudad and, because of the bending of the body axis, very slightly ventrad.

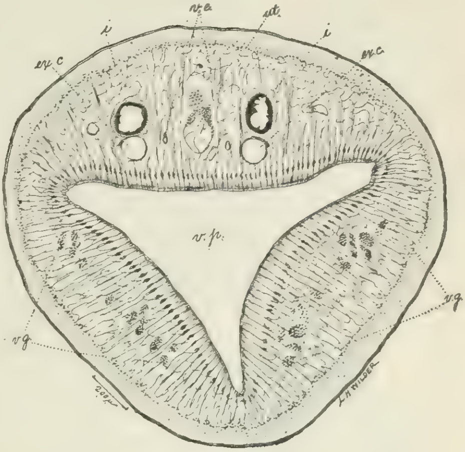


FIG. 7.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The bluntly pointed cephalic extremity is pierced by the mouth, which leads directly into a muscular oral sucker about 0.6 mm. long. This sucker lies in a large cavity (*p. s. sp.* figs. 3, 4), strongly suggestive of a rudimentary body cavity, which is traversed dorsally and ventrally by mesenterium-like bands (*m. b.* figs. 3, 4). The lumen of the sucker, in transverse section, is roughly circular near the mouth, but spindle shaped at its equator (fig. 3), with the long axis of the spindle in the transverse diameter of the animal; farther caudad this spindle becomes flattened dorso-ventrally so that the lumen becomes a transverse slit, which eventually becomes reduced to a small circular aperture as it gives entrance to the esophagus. It is provided with a number of not very prominent papillæ. The esophagus, as it leaves the base of the sucker, describes a fairly abrupt curve having its convexity ventrad and then passes caudo-dorsad to divide into two intestinal ceca; the esophagus measures about two-thirds the length of the oral sucker; dorsally of its cephalic portion is found a well-developed nerve complex.

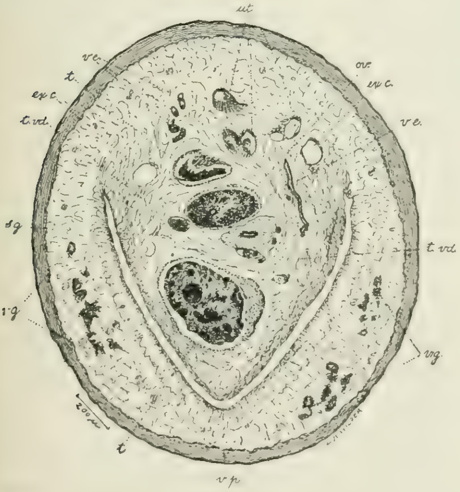


FIG. 8.

The simple intestinal ceca pass for a short distance latero-caudad, then in irregular sinuous course caudad in the dorsal body segment. They terminate at the junction of the middle with the caudal third of the body.

The lumen of the oral sucker and that of the esophagus are lined by a cuticular layer in anatomical continuation with that of the body surface. It is thin in the sucker, thicker in the esophagus, and ceases abruptly at the fork. The intestinal lumen is lined by a layer of epithelial cells.

GENITAL SYSTEM.—The two testes, the ovary, and the shell gland are in the caudal portion of the body between the fundus of the ventral pouch and the dome of the acetabulum, one testis lying dorsally, the other ventrally of the female glands.

Male organs.—The testes lie in about the same dorso-ventral plane, the left in front (ventral) of the right and separated from the latter by the interposition of the ovary, shell gland, and the dome of the distended excretory vesicle. The ventral testis extends more to the left of the median line, while the greater portion of the dorsal testis lies immediately to the right of this line, their fields consequently overlap; the latter testis lies also in a plane that is slightly lower (more caudad) than that of the former, their zones overlapping (figs. 1, 2, 8, 10). Both glands appear much shrunken, and each is made up of several lobes; the right is slightly larger than the left and the caudo-dorsal aspect of the latter appears excavated and molded to the contour of the ventro-cephalic aspect of the distended excretory vesicle (fig. 9). From the dorsal aspect of each testis there arises a vas efferens. These ducts pass dorso-cephalad, that from the left or ventral testis describing a somewhat sinuous course as it curves around to the left of the shell gland and ovary. Near the dorsum of the animal and in a plane just above (cephalad of) the testes, these ducts, one on each side of and slightly removed from the median line, turn directly cephalad and pursue a slightly sinuous course to the equator of the animal; at this level they begin to arch

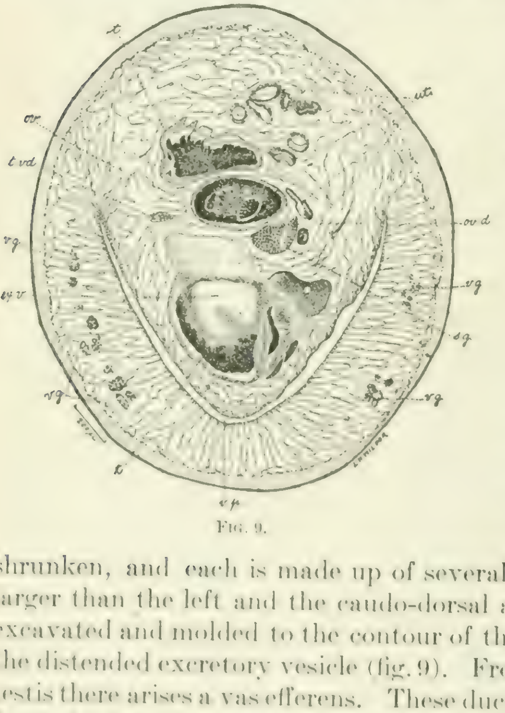


FIG. 9.

inward toward the median line and soon unite to form the vas deferens (fig. 6). In the greater part of their course cephalad they lie more or less close to the ventro-median aspect of the corresponding cecum with the uterus between them (fig. 7).

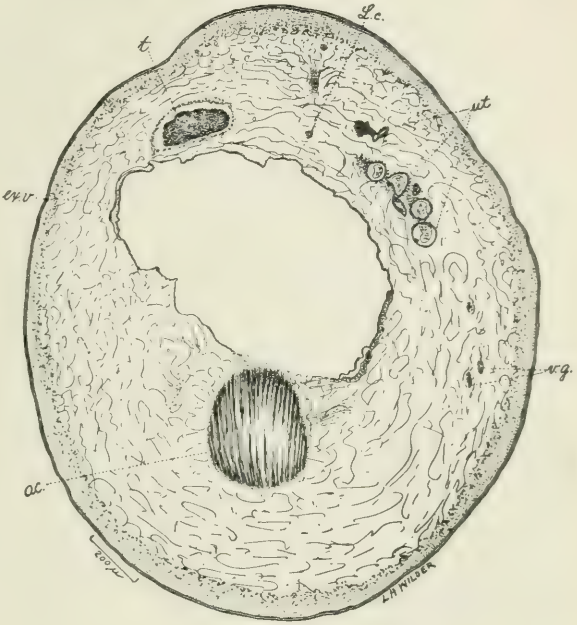


FIG. 10.

The vas deferens, almost immediately after it begins, develops a complex coil and its lumen becomes more or less dilated and filled with spermatozoa; this portion represents the vesicula seminalis. Soon its walls, which appear as delicate as those of the vasa efferentia, become abruptly thickened by an enormous increase in the muscular layers and its lumen becomes much reduced in caliber. This portion of the vas deferens (pars musculosa) continues the coil begun by the vesicula, but after pursuing a course cephalad for about twice as long a distance as that of the vesicula, the muscular wall of the duct becomes enclosed in a moderately thick layer of cells. From this point, which is about at the junction of the first with the middle third of the body, the complexity of its loops becomes very greatly reduced, its course cephalad becomes less and less winding and the thickness of its muscular wall slowly diminishes; this portion represents the pars prostatica. The coils of the vesicula, musculosa, and the windings of the first portion of the prostatica lie in the space between the corresponding portions of the intestinal ceca. As the prostatica nears the esophageal fork it tends ventrad and eventually reaches the ventral aspect of the fork at which level it describes a curve in the sagittal plane of the body with the convexity of the curve cephalad (fig. 2). At the vertex of this curve the prostatic cells abruptly cease and the duct, which at this point has become somewhat reduced in size though still with walls of considerable thickness, turns ventro-caudad, approaches and then penetrates the base of the genital papilla, at the vertex of which, having become rapidly reduced in size, it opens by a minute pore just above the aperture of the metraterm (or possibly by a pore that is common to both ducts). The single specimen available for study does not show this with sufficient clearness for definite interpretation. To the portion of the male spermatic canal beyond the prostatica the name ductus ejaculatorius may be applied.

Female organs.—Lying in the axial region of the body between the upper (cephalic) portions of the two testes are the ovary and the shell gland, the latter being close to the left ventro-lateral aspect of the former (figs. 8, 9). The superior aspect of the ovary lies in a transverse plane that is slightly below (caudad of) the superior margin of the ventral and slightly above (cephalad of) that of the dorsal testis. The oviduct springs from the ventro-caudal aspect of the left pole of the ovary at the level of the caudal aspect of the shell gland to which the duct runs and which it penetrates, bending cephalad as it does so. In the shell gland it is joined by the vitello-duct. The shell gland is an egg-shaped body, considerably smaller than the ovary, close to the left ventro-lateral aspect of which it lies, with its long axis in the transverse diameter of the body of the parasite and with the broader, more bluntly rounded pole to the left. It is penetrated by the oviduct and the vitello-duct; the former at the caudal and the latter at the left ventro-lateral aspect. The two unite and the joint duct thus formed passes transversely to the right and slightly cephalad, forming the ootype in the center of the gland. Beyond this dilatation the duct is continued as the uterus, which emerges from the right pole of the gland (fig. 8). From this point the uterus curves over to the left of the shell gland. As it skirts close to the left of the shell gland, ovary, and dorsal testis in its course to the middle line of the dorsal segment some coils dip caudad to the left of the excretory vesicle. Having reached the median line it winds its way cephalad first between the vasa efferentia then under their arch and close to the ventral aspect of the vas deferens, retaining this relation to the male genital duct in the remainder of its course and forming a curve similar to that of the pars prostatica at the level of the esophageal fork before it, as the metraterm, penetrates the genital papilla at the vertex of which it opens either just below and separate from the aperture of the male genital duct or in common with it.

The uterine canal contains relatively few eggs; these were most numerous in the portion nearest the ovary. Measurement of one of these in section gave a length of 135μ and a width of 75μ. Laurer's canal leaves the oviduct from a point close to the shell gland and then passes dorso-caudad to open by a minute pore in the dorso-median line a little below the superior margin of the acetabulum and about 0.16 mm. above the excretory pore.

The vitellaria consist of sparsely scattered follicles confined to the ventro-lateral body segments. In a vertical direction they extend from a little below the level of the genital pore to the level of the superior margin of the acetabulum. The transverse vitello-ducts pass transversely inward with a tilt cephalad to unite in the median line dorsally and slightly caudally of the superior margin of the ventral testis and just cephalad of the plane of the superior margin of the shell gland. Their point of union is not dilated into a reservoir, but a slender duct is given off which passes at first caudad for a very short distance, then to the left, skirting the ventral aspect of the shell gland which it penetrates near the left extremity and, describing a sharp curve as it does so, becomes directed to the right and unites with the oviduct.

Excretory system.—The excretory vesicle is large and distended and fills the space between the male and female sexual glands above (cephalad) and the acetabulum below (caudad) (figs, 2, 9, 10). From its dorsal aspect there springs a short excretory duct which passes caudo-dorsad to open in the dorso-median line about 0.16 mm. caudad of the pore of Laurer's canal. The duct is lined by a fairly thick cuticular layer in anatomical continuation with that of the general body surface. Two large longitudinal excretory canals are found at about the level of the esophageal fork, where they lie near the dorso-lateral aspect of the corresponding ceca. As the intestines shift latero-dorsad, the canals come to lie first close to the median side and later close to the ventro-median and ventral aspect of the ceca (figs. 6, 7). The canals pass caudad to a point in the transverse plane of the superior margin of the ventral testis, when they bend somewhat ventrad to empty into the excretory vesicle.

RELATION TO OTHER SPECIES.

This worm seems most closely related to *F. elongatus*, from which it differs chiefly in the greater proportionate length of its intestine; in the position of the ovary and shell gland, which lie between the upper portion of the testes in this form, whereas in *F. elongatus* they are caudad of the dorsal testis.

ILLUSTRATIONS.

Fig. 1.—Frontal projection showing oral sucker (*o. s.*), esophagus (*es.*), intestinal ceca (*i.*), portion of the uterus (*ut.*), the testes (*t.*), vasa efferentia (*v. e.*), and acetabulum (*ac.*). *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, *h–h*, planes of section. Enlarged. Original.

Fig. 2.—Profile projection showing oral sucker (*o. s.*), esophagus (*es.*), left intestine (*i.*), the ventral pouch (*v. p.*), the genital bulging with the genital pore leading into the genital atrium (*g. a.*), the uterus

(*ut.*), shell gland (*s. g.*), ovary (*or.*), the testes (*t.*), left vas efferens (*v. e.*), vesicula seminalis (*v. s.*), pars musculosa (*p. m.*), pars prostatica (*p. p.*), ductus ejaculatorius (*d. e.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), excretory pore (*ex. p.*), and acetabulum (*ac.*). *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, *h–h* planes of section. Enlarged. Original.

Fig. 3.—Transverse section at *a–a* figs. 1 and 2. Shows oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), and a dorsal dorso-ventral mesenterium-like strand (*m. b.*). Enlarged. Original.

Fig. 4.—Transverse section at *b–b* figs. 1 and 2. Shows aperture of ventral pouch (*a. v. p.*), papillæ on lip of aperture (*s. pap.*), oral sucker (*o. s.*), dorsal and ventral mesenterium-like strands (*m. b.*), perisuctorial space (*p. s. sp.*). Enlarged. Original.

Fig. 5.—Transverse section at *c–c* figs. 1 and 2. Shows form of body, ventral pouch (*v. p.*), ductus ejaculatorius (*d. e.*), metraterm (*ma.*), uterus (*ut.*), pars prostatica (*p. p.*), and intestines (*i.*). Enlarged. Original.

Fig. 6.—Transverse section at *d–d* figs. 1 and 2. Shows form of body, form of ventral pouch (*v. p.*), intestines (*i.*), excretory canals (*ex. c.*), the vasa efferentia about to unite (*v. e.*), uterus (*ut.*), and vitellogene glands (*v. g.*). Enlarged. Original.

Fig. 7.—Transverse section at *e–e* figs. 1 and 2. Shows form of body, form of ventral pouch (*v. p.*), position and relations of intestines (*i.*), excretory canal (*ex. c.*), vasa efferentia (*v. e.*), uterus (*ut.*), and vitellogene glands (*v. g.*). Enlarged. Original.

Fig. 8.—Transverse section at *f–f* figs. 1 and 2. Shows form of body, form of ventral pouch (*v. p.*), position and relation of the testes (*t*), ovary (*ov.*), shell gland (*s. g.*), transverse vitello-ducts (*t. vd.*), the vasa efferentia (*v. e.*), uterus (*ut.*), vitellogene glands (*v. g.*), and excretory canals (*ex. c.*). Enlarged. Original.

Fig. 9.—Transverse section at *g–g* figs. 1 and 2. Shows form of ventral pouch (*v. p.*), position and relations of the testes (*t.*), dome of excretory vesicle (*ex. v.*), ovary (*ov.*), caudal extremity of shell gland (*s. g.*), oviduct penetrating shell gland (*ov. d.*), right transverse vitello-duct (*t. vd.*), uterus (*ut.*), and vitellogene glands (*v. g.*). Enlarged. Original.

Fig. 10.—Transverse section at *h–h* figs. 1 and 2. Shows position and relations of the superior margin of the acetabulum (*ac.*), excretory vesicle (*ex. v.*), dorsal or right testis (*t.*), Laurer's canal (*L. c.*), and uterine loops (*ut.*). Enlarged. Original.

FISCHŒDERIUS SIAMENSIS, new species.

[Figs. 11 to 22.]

Specific diagnosis.—*Fischœderius* (p. 17): Body 6.6 to 15.5 mm. long; buff color (alcohol specimens); rather spindle shaped, with truncated caudal end, but most specimens distorted; greatest diameter somewhat preequatorial; oral end very bluntly pointed; longitudinal axis straight or slightly curved with concavity ventrad; transverse sections near both poles circular, but at equator nearly circular or oval to triangular with apex ventrad. Surface with coarse transverse sulci or striations, best defined near poles; otherwise smooth, except for papillæ at oral pole. Crescentic opening of pouch varies in position from oral plane to equatorial plane of oral sucker; neck of pouch with triangular to semicircular outline in transverse section, extends caudad of genital pore, body of pouch is triangular to circular in outline and extends nearly to ventral testis, but a ventral prolongation may enter testicular zone; in equatorial region, longitudinal axis of pouch coincides with axis of body. Genital pore about 0.7 mm. from aperture of pouch, either bifurcal, or slightly pre or post bifurcal; a larger external (ventral) and a smaller internal (dorsal) atrium present, the latter nearly filled by the genital papilla. Acetabulum 1.5 mm. in diameter, aperture circular, 0.6 to 1.0 mm., directed slightly ventrad. Mouth in a crateriform depression; oral sucker pyriform, 0.2 mm. long, 0.2 mm. in dorso-ventral diameter; perisuctorial space distinct; esophagus about as long as oral sucker; bifurcation about on plane of genital pore; ceca short, extend to equator or slightly beyond, tortuous, in longitudinal dorsal body segment. Excretory pore dorso-median or right or left, about on plane of cephalic margin of acetabulum, caudad of pore of Laurer's canal.

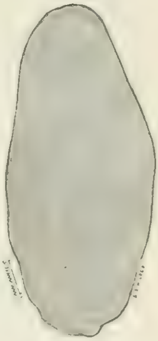


FIG. 11.

Male organs: Testes median, one dorsal of the other, markedly indented, fields and zones overlap, immediately preacetabular; vasa efferentia unite in preequatorial body zone about at junction of cephalic and equatorial thirds; vesicula and musculosa coiled, prostatica sinuous; ductus ejaculatorius opens separately from metraterm.

Female organs: Ovary in testicular zone and field, dorso-caudad or caudad of dorsal testis, median, right, or left of median line; vitellaria chiefly in ventrolateral longitudinal body segments, but to some extent in dorsal segment lateral of ceca, extending about from plane of genital pore to or near the first testis; uterus extends from shell gland dorso-lateral, may dip caudad, turns cephalad, runs dorsally of testes, under union of vasa efferentia, in suctorial field to genital papilla, discharging separately from ductus ejaculatorius; Laurer's canal extends from oviduct in sinuous course cephalo-dorsad to dorsum, and discharges median or right or left, in plane slightly cephalad or caudad of cephalic margin of ovary.

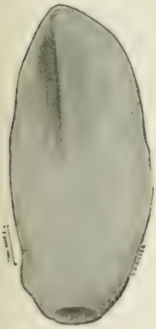


FIG. 12.

Eggs: Not measured.

HABITAT.—In (? organ of) "Cow" (*Bos* sp.), Phrapatoom Siam.

TYPE.—U.S.P.H.& M.H.S. 9956.

SOURCE OF MATERIAL.—These worms form part of a sending by Dr. P. G. Woolley from Phrapatoom, Siam. The host was given as *Bos* ("cow").

EXTERNAL CHARACTERS.

SIZE.—Measurements of 28 specimens show considerable variation in size. The length varies from 6.66 mm. to 15.5 mm.; in only 7 of 28 specimens does the length exceed 10 mm.

COLOR.—The worms (fixed, and preserved in glycerine alcohol) are of a buff color.

FORM.—Most of the specimens appear more or less distorted; only a few appear to have preserved what is presumed to be their natural shape. These show considerable variation (figs. 11–14) in form; in general, however, this is not unlike a spindle with a truncated caudal extremity. The greatest diameter of this spindle form is not in the equatorial region of the worm but somewhat preequatorial. The cephalic extremity is very bluntly pointed. The longitudinal axis is straight or very slightly curved with concavity of the curve ventrad. The caudal extremity is truncate; it presents the aperture of the acetabulum, the plane of which, being directed with a certain obliquity from the venter to the dorsum and downward, appears to be tilted more or less ventrad.

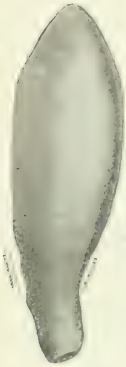


FIG. 13.

The outline of the worm in transverse section is approximately circular both at the caudal and the cephalic extremities. In the equatorial region, however, some specimens show a triangular, some a triquadrant, and others a more or less circular or oval form. When triangular, the apex of the triangle is directed ventrad. This ventrally directed angle is replaced by a vertical groove in those specimens presenting a triquadrant form.

SURFACE.—The general cuticular surface is marked by coarse transverse striations or sulci, that are best defined about the cephalic and the caudal extremities; it is smooth otherwise except at the cephalic pole where it is beset by short, more or less conical papillæ which are most numerous and most prominent immediately around the oral aperture. The papillæ measure up to 0.03 mm. in length and up to 0.045 mm. in width at the base.

Ventral pouch.—The position of the aperture of the pouch varies; in some it is in the same transverse plane as the mouth (fig. 16); in others it is somewhat caudad of this plane, namely, about in the equatorial plane of the oral sucker (fig. 15).

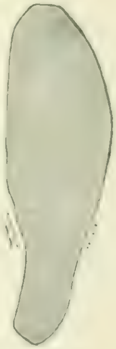


FIG. 14.

The aperture is followed by a passage the dimensions of which increase but slowly up to the level of the genital pore; beyond this its dimensions increase rapidly and soon this passage, which may be regarded as the neck, expands into the body of the pouch. The pouch extends caudad to a point in a transverse plane slightly cephalad of the ventral testis, though in some a more or less crescentic slit-like prolongation is tucked in, as it were, ventrally of this gland.

The fundus of the pouch is in a plane directed more or less obliquely from the dorsum ventrad and caudad so that the dorsal wall of the pouch is shorter than the ventral.

The dorsal wall of the neck of the pouch is arched ventrad, relatively slightly in the vertical, but considerably in the transverse direction. It differs markedly, however, from the semipedunculated globular bulging in *F. fischœderi* (fig. 2).

The form of the pouch in transverse sections depends somewhat on the level. The aperture is a simple transverse crescentic slit (fig. 15). At the level of the genital pore the form of the neck of the pouch is or tends to a triangular outline with the apex ventrad (fig. 17). In one of the specimens, however, the outline approached more nearly that of a semicircle, with the arch ventrad.

In the equatorial region of the animal the outline of the pouch varies in different individuals. In some it is triangular; in others it is almost circular, though in some of the latter specimens the pouch may present a triangular outline in a plane farther cephalad.

At the level of the cephalic aspect of the dorsal testis the outline of the pouch in transverse section also varies considerably in different specimens; in some it is a crescentic slit; in others, a caret-like slit, and in still others more or less irregularly circular.

In the equatorial region of the animal the longitudinal axis of the pouch coincides substantially with the longitudinal axis of the body. Whatever the form of the pouch in transverse section, the encircling body is divided into three approximately equal longitudinal segments by constrictions; one of the latter occupies the median ventral line and the others the right and left dorso-lateral regions, respectively (fig. 21). These constrictions correspond in position to the angles of the triangle when the pouch has this form.

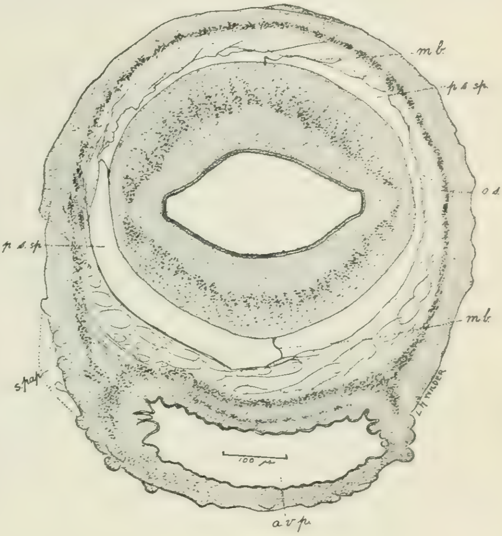


FIG. 15.

Genital pore.—On the dorsal wall of the pouch, about 0.70 mm. from the aperture, is the genital pore. It is at about the level of the esophageal fork in 3 of 7 specimens; in 2 it is above and in 2 slightly below this level. The pore gives entrance to an atrium which appears to be divided into 2 chambers by a ring-like projection of the atrium wall. Of the 2 chambers thus formed, the outer, or ventral, is much the larger. The inner, or dorsal, is almost completely filled by the genital papilla, leaving only a narrow slit-like space around the latter. The genital papilla projects into the chamber from what would be its dorsal wall. At the vertex of the papilla is the crater-like orifice of the metraterm and immediately above the latter is the minute aperture of the ductus ejaculatorius (figs. 17, 18, 19).

The projecting ring on the atrium wall varies considerably in prominence. In some specimens it is very well marked; in others it is but ill defined and apparently due only to a slight fold or narrow groove in the atrium wall.

Acetabulum. This excavated somewhat hemispherical muscular organ occupies the caudal portion of the body. The terminal aperture is circular, tilted slightly ventrad, and in two sectioned specimens measured 0.6 mm. and 1.00 mm. in diameter, respectively.

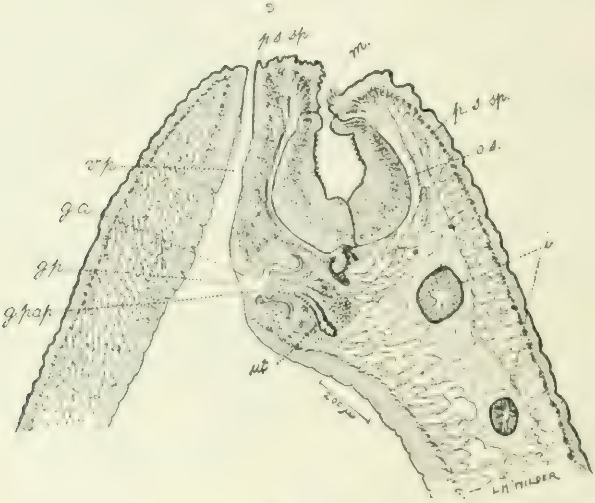


FIG. 16.

The muscular dome measured 0.45 mm. and 0.34 mm., respectively, in thickness in these two sectioned specimens.

The greatest diameter of the acetabulum measured in sectioned specimens was 1.5 mm. in one and 1.17 mm. in another.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The vertex of the bluntly pointed cephalic extremity is more or less depressed in the form of a more or less shallow irregularly circular crater, at the bottom of which is the mouth of the animal. This leads directly into a muscular pyriform oral sucker. The latter lies in a distinct cavity (figs. 15, 16), which is crossed dorsally and ventrally by dorso-ventral strands. Besides these strands, the sucker is attached at both its poles to the body parenchyma. The lumen of the sucker varies somewhat in form in different individuals. In a general way it is a transversely broad, but dorso-ventrally a rather narrow space. In transverse sections the outline of the lumen varies at different levels. In the region of the mouth it is more or less circular or elliptical; farther caudad, about in the plane of the equator of the sucker, the outlines become spindle shaped with the major axis of the spindle in a transverse direction. This spindle-shaped outline is rapidly reduced to a transverse slit, and the slit in its turn, as the result of a contraction of its transverse diameter, becomes reduced to a small circular aperture which leads into the esophagus. The lumen is lined by a thin cuticle which, over about the cephalic half, is beset by very minute conical papillæ. The esophagus springs from the base of the oral sucker, and for a very short distance passes caudad in the axis of the latter; then rather abruptly it turns dorsad with but a slight inclination caudad. Its length is about equal to but does not exceed that of the sucker and it divides, laterally, into two intestinal cecal tubes. The angle formed by the fork appears to be somewhat in excess of 90°. The lumen is lined by a thick, cuticular layer.

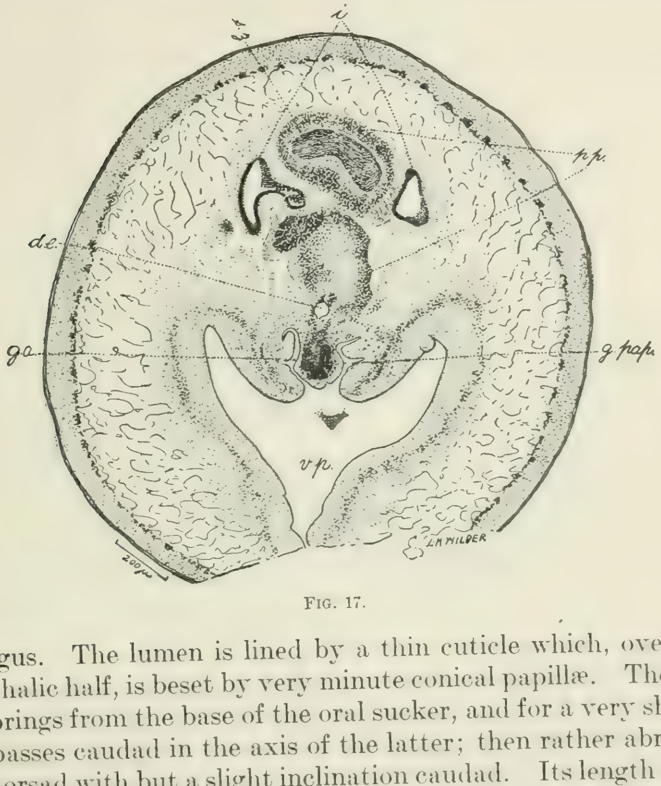


FIG. 17.

The intestinal ceca, from their point of origin, pass, at first, latero-caudad, then directly caudad to terminate about midway between the cephalic and the caudal extremities. The ceca may extend slightly beyond this point, or they may fall distinctly short of it. They pursue a somewhat tortuous course in the dorsal body segment from one-fourth to one-third of the width of the segment mediad of the constriction marking off this segment from the others. The lumen of the esophagus is lined with a cuticle-like layer, which ceases abruptly at the fork. Here it is replaced by a nucleated cell layer which extends throughout the intestine.

Genital system.—*Male organs:* The testes lie in the caudal portion of the axial region of the body immediately preacetabular (fig. 22). One is more or less directly dorsad of the other, but in slightly different though overlapping zones. The dorsal of the two testes is, as a rule, the one found in the higher (cephalad) of the testicular zones.

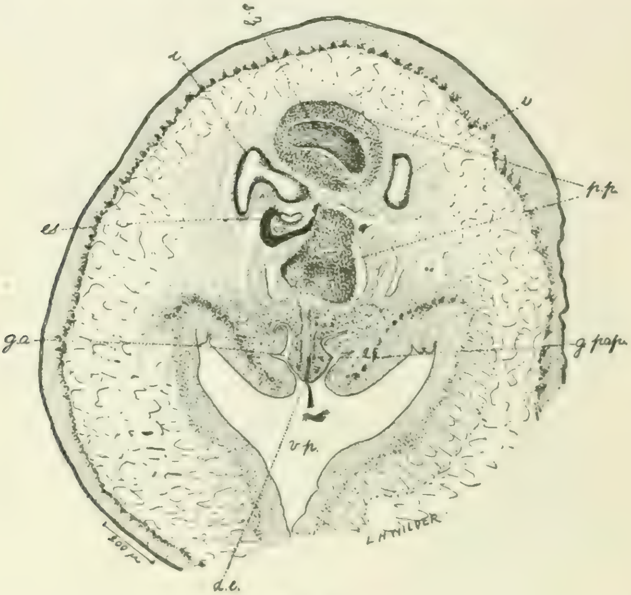


Fig. 18.

As a rule the testis from which the right vas efferens springs (the [?] right testis) is also the dorsal (and cephalic) one. In two of eight specimens studied this arrangement was reversed, the left testis (namely, the testis from which the left vas efferens springs) being dorsad and slightly cephalad of the right.

The margins of the testes are markedly indented, the indentations marking off lobes of different dimensions. From the dorsal or dorso-cephalic aspect of each testis there springs a vas efferens which passes dorsad and slightly cephalad. The vas from the ventrally placed of the two testes, usually the left, describes a curve outward in its course as it skirts the margin of the dorsally placed gland. At about the level of the superior aspect of the superior testis the vasa efferentia change their course and proceed directly cephalad in the dorsal longitudinal body segment more or less close to and one on each side of the uterus. At about the level of the equatorial plane of the body of the animal the vasa efferentia come into relation with the intestinal ceca close to the ventro-median aspect of each of which they then continue on their way cephalad. They maintain this relation for some distance, then they bend rather abruptly inward toward the median line to unite and form the vas deferens. This union takes place at about the junction of the anterior with the middle third of the body.

The vas deferens, immediately after it begins, develops coils as it winds its way cephalad. These coils show a lumen which is more or less dilated in different specimens with walls no thicker than those of the vasa efferentia. This is the vesicula and, after proceeding a short distance, its walls become abruptly thickened by a marked increase in the muscular layer. This portion is the pars musculosa which also forms coils as it proceeds cephalad. These coils have a lumen that in some specimens is little if any smaller than that of the vesicula. This pars musculosa proceeds cephalad to within a short distance of the esophageal arch, where its direction is changed to cephalo-ventrad. At about this point, too, it ceases to form coils and is continued as the pars prostatica of the vas deferens. The pars prostatica pursues a more or less sinuous course and is inclosed in a thick layer of cells with well-defined nuclei. On reaching the base of the genital papilla this cell layer abruptly ceases. Before this point is reached there is ob-

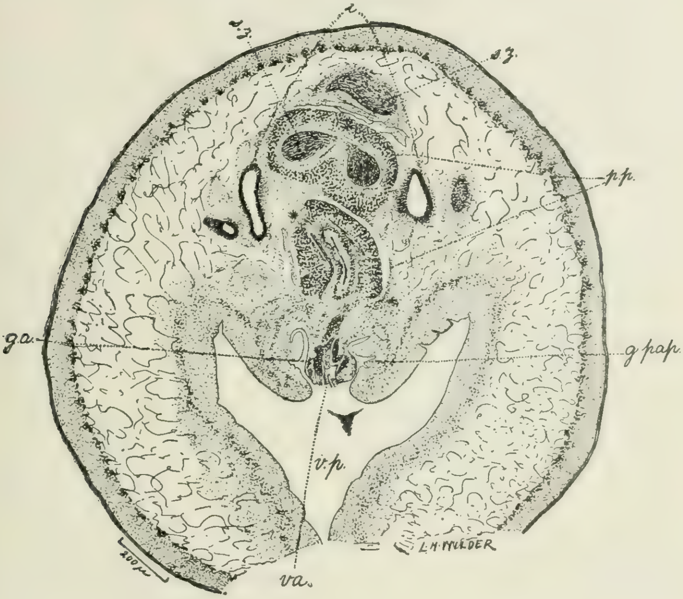


FIG. 19.

servable also a gradual reduction in the thickness of the muscular coat. After penetrating the genital papilla, the wall of this duct, which now corresponds to the ductus ejaculatorius of other forms, becomes progressively thinner and its caliber becomes rapidly reduced in diameter so that at the vertex its opening is but a minute slit above the large female aperture.

Female organs.—The ovary lies in the testicular zone, dorso-caudad or directly caudad of the dorsally placed testis whether the latter be the left or the right. In other respects, however, its position is subject to considerable variation. In four of seven specimens the ovary was to the right, though close to the median dorso-ventral line; in two it was found in the median line, while in one it was to the left of this line.

The shell gland lies close to the ovary, but in other respects its relation to the latter varies considerably. In five of eight specimens the shell gland was found close to the left ventro-caudal aspect of the ovary, in two it was close to the right ventro-caudal aspect, and in one instance it was directly ventrad of the ovary.

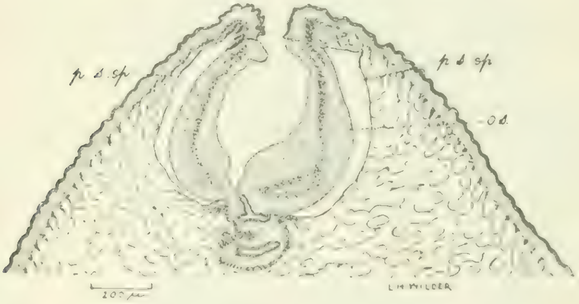


FIG. 20.

The oviduct springs from the ovary and passes to the shell gland, which it penetrates. Variations were observed both as regards the aspect of the ovary from which the duct sprang and the path which it pursued in its course to the shell gland. In six of eight specimens the oviduct sprang from the left lateral aspect of the ovary, in one from the right, and in one from the caudal aspect.

The path which the oviduct describes is more or less curved and is either in a transverse (horizontal) or in a more or less vertical plane.

The uterus emerges from the ventral, right or left lateral, or cephalic aspect of the shell gland. In none of the specimens studied did it emerge from either the dorsal or the caudal aspect. From this the uterus passed to the left dorso-lateral area of the body in seven of the eight specimens studied by us; in one instance the uterus passed to the right dorso-lateral region. In these areas the uterus at first may dip somewhat caudad, then it turns dorso-cephalad and toward the median line, passing dorsally of the dorsal testis and of the right or the left vas efferens, according as the uterus occupied the right or the left dorso-lateral areas. Having reached the median dorsal line it pursues a zigzag winding course cephalad with a vas efferens on each side and passes beneath the arch formed by their union to gain the ventral aspect of the coiled vas deferens. It maintains this relation in the remainder of its course cephalad and ventro-cephalad to the genital papilla, which it penetrates. In this latter portion of its course the zigzag windings have become markedly reduced. Its terminal portion, the metraterm, pierces the genital papilla and opens by a large crateriform pore at the vertex of the latter.

The uterus contains a few eggs and the first portion also some spermatozoa; in one case it contained large cells resembling germ cells. Laurer's canal leaves the oviduct a little before the latter penetrates the shell gland. It then passes to the dorsum of the animal, describing a more or less sinuous course cephalo-dorsad to the right or left over or beneath the ovary to its pore on the dorsal surface; the pore lies in the median line or more or less to the right or left of it and in a transverse plane, which may be slightly above or below the superior margin of the ovary. In seven of eight specimens this point was more or less above the superior margin of the acetabulum, while in the eighth the pore of Laurer's canal was in a plane slightly below the superior margin of the acetabulum.

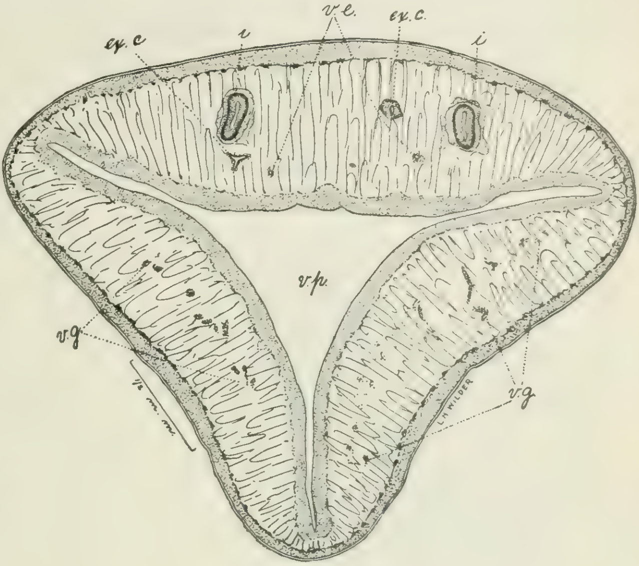


FIG. 21.

The vitellaria consist of sparsely scattered somewhat globular follicles, for the most part confined to the ventro-lateral body segments, but encroaching to some extent on those portions of the dorsal body segment external to the line of the intestinal ceca. They extend from about the level of the genital pore to a little short of or occasionally partly overlapping the zone of the superior testis. The transverse vitello-ducts pass mediad and slightly caudad from the lateral regions of the body about at the level of the ovary and ventrad of the latter to unite at the right or the left of and close to the shell gland, at which point a somewhat triangular dilatation is formed. From the dorsal aspect of this dilatation (? vitelline reservoir) a relatively slender vitello-duct passes toward the shell gland and skirts one of the caudo-lateral aspects of the latter; on reaching the dorso-lateral aspect of this gland it curves cephalo-ventrad to penetrate the gland and then unites with the oviduct in the formation of the ootype.

EXCRETORY SYSTEM.—In the specimens studied the excretory vesicle was collapsed or only slightly distended and was placed dorso-cephalad of and at about the level of the dome of the acetabulum. The excretory duct leaves the dorsal aspect of the vesicle and passes to the dorsum in a more or less sinuous course, in most cases with a slight tendency caudad, but in some horizontally, and in others with a slight tilt cephalad. The excretory pore is caudad of the opening of Laurer's canal and is about at the level of the superior margin of the acetabulum, though in none of the specimens was it exactly so; in three of eight specimens it was slightly above and in five slightly below this point. Usually, also, it is in about the median dorsal line, but we found it somewhat to the right in one instance and considerably to the left in another. In each of two specimens it measured about 30μ in diameter.

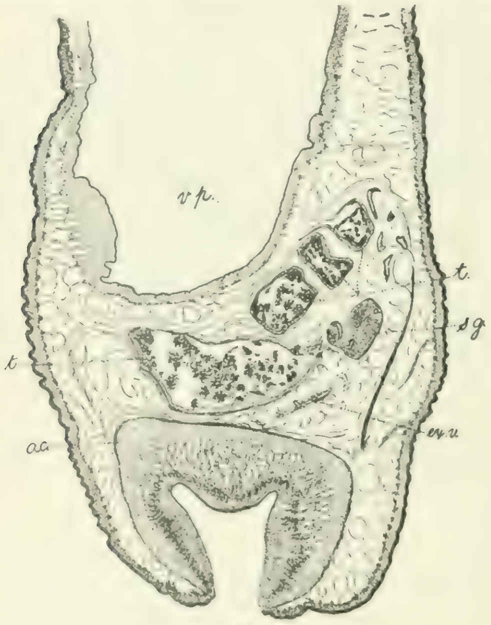


FIG. 22.

There are two main longitudinal canals which pass caudad in the dorsal longitudinal body segment lying near and ventral of the intestinal ceca in the cephalic half of the body, and they maintain this relative position beyond the termination of the ceca to the excretory vesicle.

ILLUSTRATIONS.

FIG. 11.—Ventral view. Enlarged. Original.

FIG. 12.—Profile view of specimen shown in Fig. 1. Enlarged. Original.

FIGS. 13 and 14. Profile views to show variation in form. Enlarged. Original.

FIG. 15.—Transverse section at level of aperture of ventral pouch. Shows form of body, surface papillæ (*s. pap.*), form of aperture of

ventral pouch (*a. v. p.*), form of oral sucker and its lumen (*o. s.*), perisuctorial space (*p. s. sp.*), and dorsal and ventral mesenterium-like strands (*m. b.*). Enlarged. Original.

FIG. 16.—Sagittal section through oral extremity. Shows mouth (*m.*), oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), aperture and neck of ventral pouch (*v. p.*), genital pore (*g. p.*), genital atrium (*g. a.*), genital papilla (*g. pap.*), intestine (*i.*), and uterus (*ut.*). Enlarged. Original.

FIGS. 17, 18, and 19.—Series of three transverse sections. Show form of body, form of neck of ventral pouch (*v. p.*), knuckle of esophagus (*es.*), intestinal ceca (*i.*), pars prostatica (*p. p.*) containing mass of spermatozoa (*sz.*), separate openings of ductus ejaculatorius (*d. e.*) and metraterm (*va.*), genital papilla (*g. pap.*) and genital atrium (*g. a.*). Enlarged. Original.

FIG. 20.—Frontal section through oral extremity to show sucker (*o. s.*) and perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 21.—Transverse section to show form of body and of ventral pouch (*v. p.*) in equatorial region. Shows also excretory canal (*ex. c.*), intestines (*i.*), vasa efferentia (*v. e.*) and vitellaria (*vg.*). Enlarged. Original.

FIG. 22.—Sagittal section of caudal extremity showing relations of acetabulum (*ac.*), excretory vesicle (*ex. v.*), testes (*t.*), shell gland (*s. g.*), and fundus of ventral pouch (*v. p.*). Enlarged. Original.

FISHŒDERIUS CEYLONENSIS, new species.

[Figs. 23 to 32.]

SPECIFIC DIAGNOSIS.—*Fischœderius* (p. 17): Body 6 to 7 mm. long by 2.56 mm. broad by 2.52 mm. thick; buff color (alcohol specimen); rather conical, but greatest diameter about at junction of equatorial and caudal thirds; rather prominently attenuated cephalad, slightly attenuated caudad; longitudinal axis slightly curved, concavity ventrad; cephalic end bluntly pointed; caudal extremity rather truncate; dorsum somewhat convex, venter slightly concave; lateral margins convex longitudinally; transverse sections near both poles nearly circular, but in equatorial region triangular with rounded angles, apex ventrad. Surface smooth, with exception of a few papillæ around mouth and aperture of ventral pouch. Opening of ventral pouch 0.62 mm. from oral margin; pouch begins with a narrow neck which extends to caudal margin of genital atrium (ventral chamber), then dilates into cavity which extends along dorsal wall to cephalic margin of testes and along ventral wall to equator of acetabulum; transverse sections of aperture and neck crescentic, and of cavity rather triangular with apex ventrad. Genital pore 1.18 mm. from cephalic margin, somewhat prebifurcal, in esophageal zone; a large external (ventral) and a small internal (dorsal) atrium present. Acetabulum 1.6 mm. in transverse and about 1 mm. in dorso-ventral diameter, terminal, slightly sunken in body, its 0.46 mm. aperture directed caudad and because of curvature of body axis very slightly ventrad. Mouth terminal, leads directly into oral sucker which is rather elongate pyriform, 0.62 mm. long, 0.33 mm. broad, 0.3 mm. thick, and lies in a well-developed cavity which is traversed dorsally and ventrally by mesenterium-like bands; esophagus relatively long, 0.72 mm., hence longer than oral sucker; bifurcation at junction of first and second

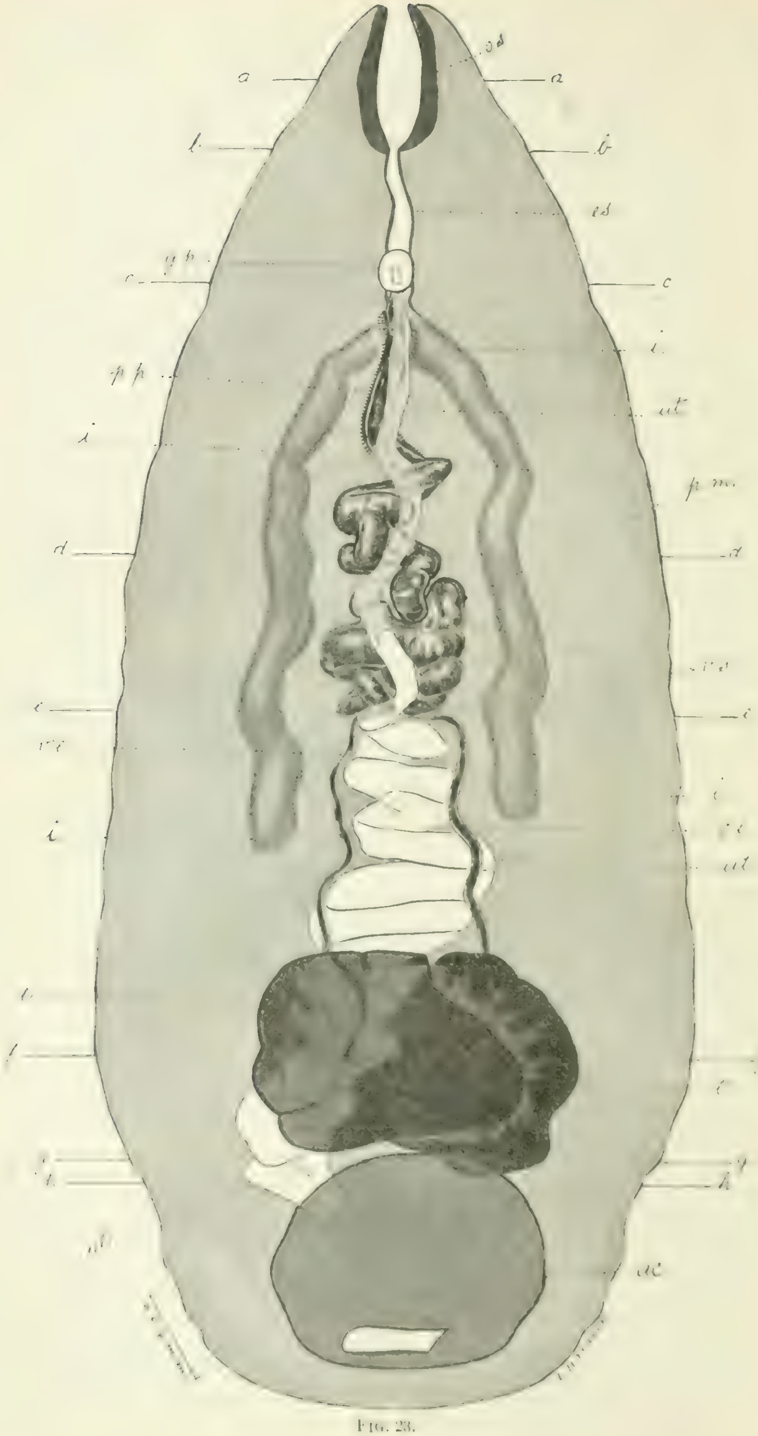
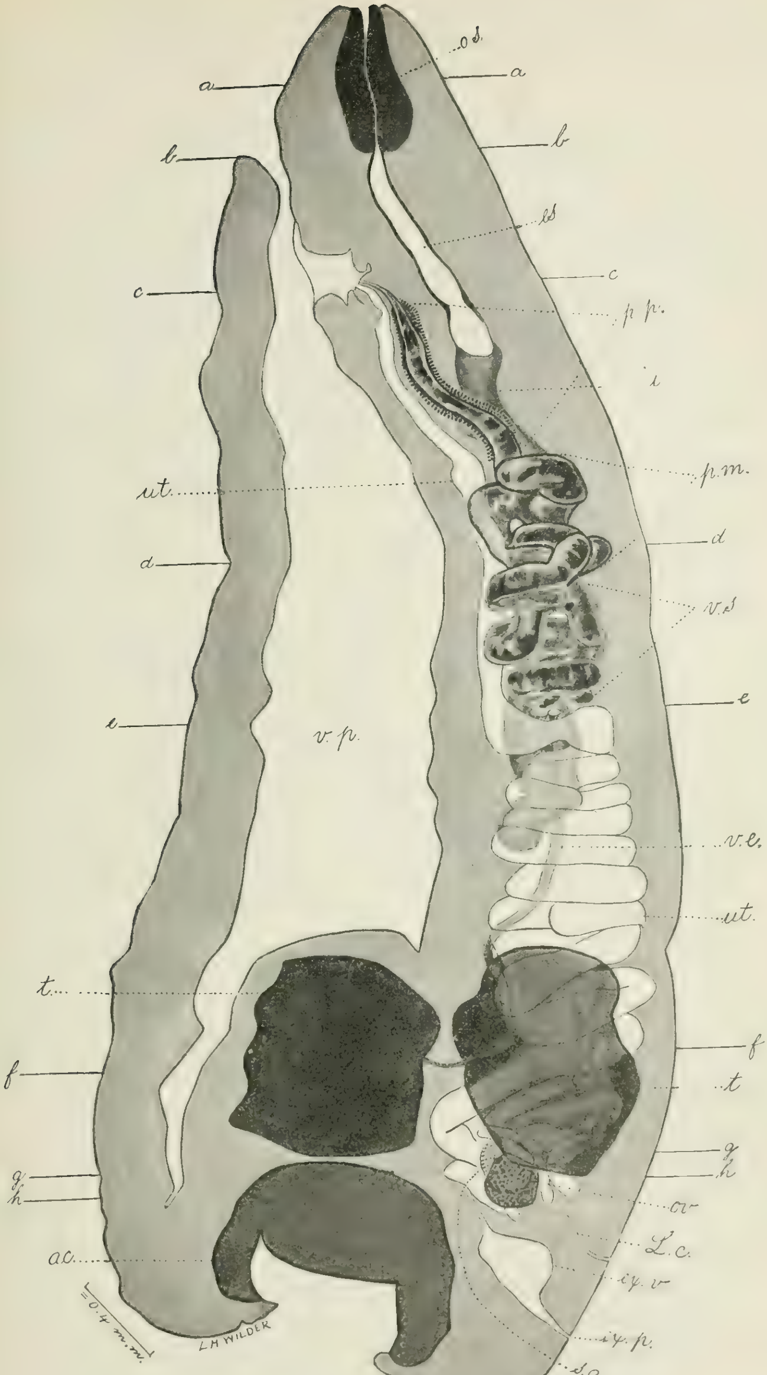


FIG. 23.


o.s.
a
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f
f
t
g
h
g
h
ov
L.c.
ac.
i4.v
i4.p.
s.g.
= 0.4 m.m.
L.H. WILDER

FIG. 24.

fourths of body, ceca short, extend about to junction between third and fourth fifths of body. Excretory pore dorso-median about at equator of acetabulum and slightly (0.36 mm.) caudad of pore of Laurer's canal; duct short, vesicle not much distended in type specimen.

Male organs.—Testes large, indented, one dorsal of the other, immediately pre-acetabular, median, zones coincide, fields overlap, one testis extending more to the right, the other more to the left, of median line; vasa efferentia unite at equator of body; vesicula and pars musculosa well defined and coiled; pars prostatica long, nearly straight; ductus ejaculatorius relatively short, but distinct, discharges separately from metraterm.

Female organs.—Ovary and shell gland ventro-caudal of and very much smaller than dorsal testis; vitellaria in ventro-lateral and dorsal body segments, lateral of ceca, extending from level of caudal end of pars prostatica into but not beyond equatorial third; uterus extends from shell gland dextrad, dips slightly caudad, turns cephalad, forming loops dorsally of ventral and to right of dorsal testis, expands, passes under arch of vasa efferentia, then in slightly irregular line, but without loops, it extends cephalad in suctorial field ventrally of vas deferens, the metraterm discharges just below ductus ejaculatorius into atrium; Laurer's canal extends from oviduct dorso-caudad to dorso-median line 0.36 mm. cephalad of excretory pore and about on plane of cephalic margin of acetabulum.

Eggs: Not observed.

HOST.—*Bos kerabau*, apparently from Ceylon, died in zoological garden in Germany.

TYPE.—U.S.B.A.I. 15358.

SOURCE OF MATERIAL.—The single specimen studied was taken from a bottle numbered 3376, containing a label with the following legend:

> Name, *Gastrothylax synethes* Fischœder. Cotype. Host. *Bos kerabau*. Locality, Kœnigsberg, Tiergarten [Leipzig]. Collected by Fischœder. Determined by Fischœder. Presented by Luehe; date, June, 1902.

The specimen, after staining with carmine, dehydration, and clearing, was found to differ from *Gastrothylax synethes* in several respects. It was then numbered 15358, sectioned, and studied in detail.

EXTERNAL CHARACTERS.

SIZE.—The alcohol specimen measured 7 mm. in length and 3 mm. in greatest width. Measurements from sections give 5.98 mm. length, 2.56 mm. greatest width, and 2.52 mm. greatest dorso-ventral diameter.

COLOR.—The worm was of a buff color.

FORM.—In a general way this specimen resembles both *G. synethes* and *G. elongatus*. Its greatest transverse and dorso-lateral diameters are at about the junction of the equatorial with the caudal third of the body. From this region toward the poles these diameters become progressively reduced, markedly toward the oral, slightly toward the aboral pole.

The longitudinal axis is slightly curved with the concavity ventrad.

In transverse section the outline of the body near the oral and aboral extremities is substantially circular (figs. 25–27, 30–31), though just above the aperture of the ventral pouch the venter is flattened. In the equatorial region (figs. 28, 29) it is triangular in form with rounded angles, one of which occupies the median ventral longitudinal line.

Surface.—The surface cuticle is unarmed, but there are a few minute papillæ at the oral pole immediately around the mouth and on the lip of the aperture of the ventral pouch (fig. 26).

Ventral pouch.—On the ventral aspect of the animal, 0.62 mm. (measured from sections) caudad of the oral margin, there is a transverse depression which is the aperture of the ventral pouch (figs. 24, 26). This aperture leads into a ventro-dorsally narrow, slit-like passage which may be regarded as the neck of the pouch. This extends caudad to the lower (caudal) margin of the ventral chamber of the genital atrium, caudally of which it rapidly dilates into the body of the pouch. The body of the pouch extends caudad to just above the plane of the superior margin of the ventral testis. The fundus of the pouch (as in *F. fischœderi*) bulges somewhat cephalo-ventrad into the lumen in such a manner as to make the dorsal wall of the pouch much shorter than the ventral, and the lateral walls intermediate gradations between the two. As a result of this there is (in transverse sections) a crescentic slit-like prolongation of the pouch caudad between the ventral testis and acetabulum, on the one hand, and the ventral wall of the worm on the other (figs. 24, 30, 31). It extends caudad to a little below the superior margin of the acetabulum.

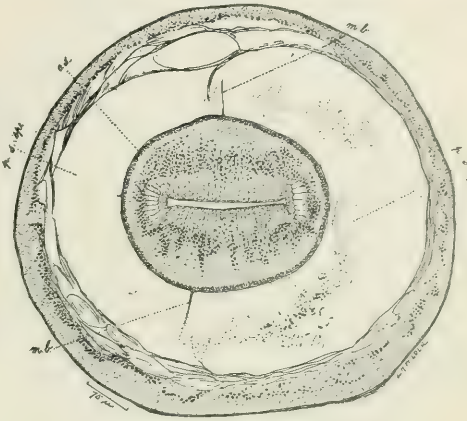

Fig. 25.

The outline of the pouch in transverse sections varies at different levels; the aperture is a narrow crescent measuring 0.135 mm. (in sections) in dorso-ventral diameter, with rounded horns, the distance between which is 0.525 mm. (in sections); the neck of the pouch retains the crescentic outline of the aperture; in the equatorial region of the worm the body of the pouch is somewhat triangular in outline with the apex directed ventrad. Corresponding to the position of the angles of this triangle the inclosing body shows constrictions which divide it into three segments, one dorsal and two ventro-lateral, of which the former is somewhat the largest (figs. 28, 29).

In the equatorial zone the pouch occupies substantially the axial region of the animal.

Genital pore.—On the dorsal wall of the neck of the ventral pouch there is a longitudinally elliptical opening, the genital pore, measuring 0.40 mm. in length by 0.30 mm. in width, leading into a large somewhat circular depression measuring 0.20 mm. in greatest depth (figs. 23, 24, 27). At the bottom of this depression (ventral chamber of genital atrium) is a small pore about 0.05 mm. in diameter leading into the slit-like dorsal chamber. The dorsal wall of this is formed by the genital papilla. At the vertex of the latter may be seen the minute aperture of the ductus ejaculatorius, and immediately beneath this, the considerably larger opening of the metraterm. This papilla is just above (cephalad of) the level of the esophageal fork.

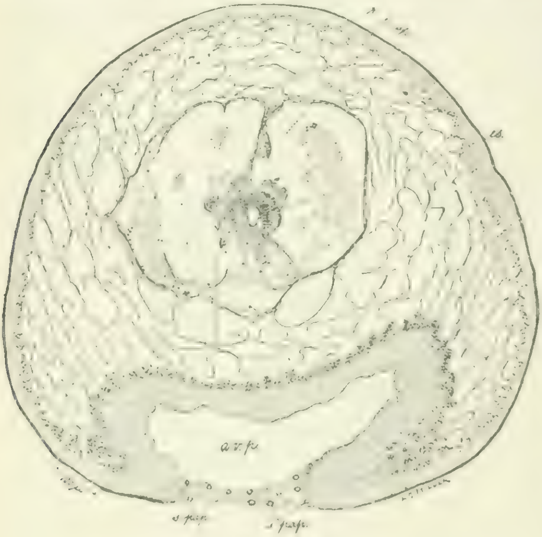

FIG. 26.

Acetabulum.—This organ of adhesion, measuring about 1.16 mm. in transverse and about 1 mm. in dorso-ventral diameter, occupies the caudal terminal portion of the body, and its aperture, directed downward (caudad) and, because of the curvature of the body axis, slightly ventrad, measures about 0.46 mm. in diameter.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The bluntly pointed cephalic extremity is pierced by the transversely elongated spindle-shaped aperture of the oral sucker (the mouth). The sucker consists of a dorsal and a ventral muscular mass continuous laterally (figs. 23–25). The sucker measures 0.62 mm. in length, 0.30 mm. in greatest dorso-ventral, and 0.33 mm. in greatest transverse diameter. Its lumen is a narrow transverse slit, which becomes reduced to a minute aperture at the opening into the esophagus. The sucker rests in a roomy space (*p. s. sp.*, figs. 25, 26), in which it is held in position by its anchorage to the parenchyma and cuticle at its oral extremity, by the esophagus at its caudal extremity, and between these points by dorsal and ventral mesenterium-like strands.

The esophagus begins at the base of the sucker and passes caudad with but a slight tilt dorsad. It measures 0.72 mm. in length, and at the junction of the first with the second fourths of the body length it forks into the two intestinal ceca. The latter pass at first (for about one-fourth of their length) latero-caudad then in a direct though slightly sinuous course directly caudad in the dorsal body segment to terminate by cecal extremities at about the junction of the third with the fourth fifth of the body length. The cecal extremities lie, however, in slightly different transverse planes, the left being slightly above the right. As in *F. elongatus*, the lumen of the sucker and that of the esophagus are lined by a cuticle in anatomical continuation with that of the body surface; it is thin in the sucker, thicker in the esophagus, and ceases at the bifurcation. The intestinal ceca are lined throughout by a layer of epithelial cells.

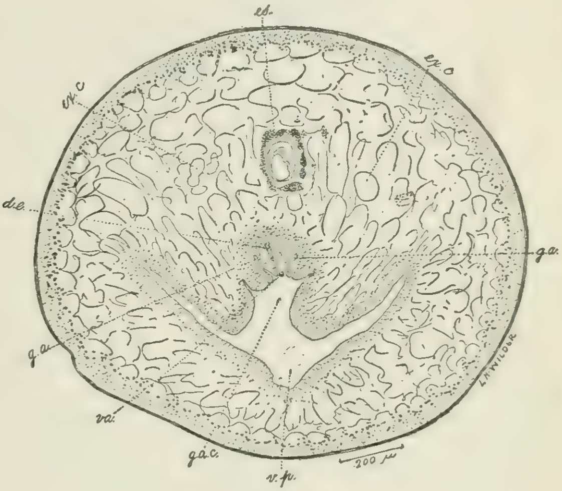

FIG. 27.

GENITAL SYSTEM.—The testes, the ovary, and the shell gland are in the caudal portion of the body immediately cephalad of the acetabulum.

Male organs.—The two large testes lie in the same dorsoventral line, the left dorsad of the right. The bulk, however, of the left testis lies to the left of the median line while that of the right or ventral testis lies to the right of the median line, so that their fields overlap. Both testes show marked indentations. A vas efferens springs from the dorsal aspect of each testis (fig. 30). From its point of origin the left vas efferens passes almost directly cephalad in the dorsal body segment a little to the left of the median line. The right vas efferens, on account of the difference in the position of the testes, is longer and at first runs almost horizontally dorsad but very soon turns and runs directly cephalad a little to the right of the median line. The two vasa efferentia unite in the median line in the equatorial plane of the body. Just before they unite each vas describes a curved course mediad so that by their union they form a transverse arch (figs. 29, 23). In their course the vasa are separated one from the other by coils of the uterus and their distal portions run more or less close to the ventromesial aspect of the terminal portions of the corresponding intestinal ceca.

The vas deferens, formed by the union of the vasa efferentia, shows a well-marked, thin-walled vesicula seminalis, a well-defined pars musculosa, 127μ in diameter with walls 37μ in thickness (both portions very much coiled) and a long pars prostatica. The latter pursues an almost direct course cephalo-ventrad beneath, and then ventrally of the esophageal fork and caudal portion of the esophagus to the base of the genital papilla. At this point the prostatic cells enclosing the pars prostatica cease and the continuation of the duct pierces the genital papilla and opens by a minute aperture at the vertex of the latter. This terminal portion of the male canal becomes very rapidly thin walled and greatly reduced in caliber and corresponds to the ductus ejaculatorius.

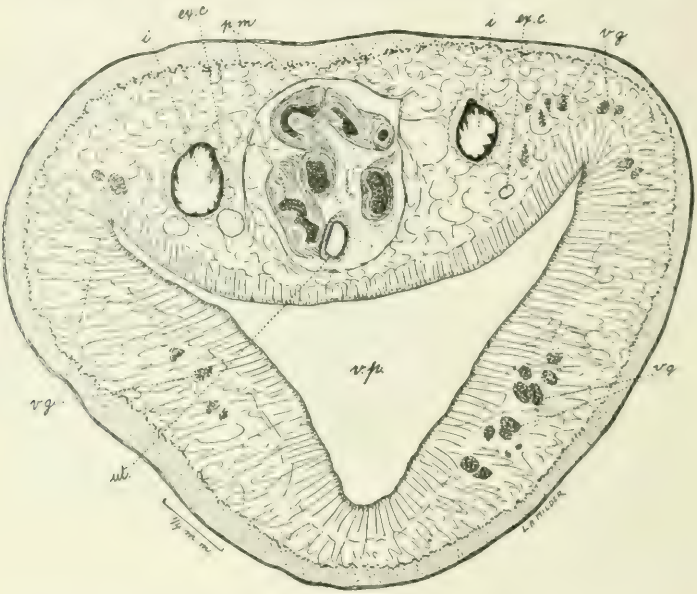

FIG. 28.

Female organs.—The ovary and shell gland lie obliquely side by side, close to the ventro-caudal aspect of the dorsal (left) testis (figs. 31, 24). The ovary lies obliquely to the left of the shell gland and gives origin to the oviduct from its right lateral aspect. The oviduct at first passes slightly caudad from its point of origin, then turns abruptly to the right and cephalad to penetrate the dorso-caudal aspect of the shell gland, within the body of which it is joined by the vitelloduct and forms the ootype. The shell gland lies close to the right cephalo-mesial aspect of the ovary. It is penetrated, as already described, by the oviduct and a little to the right of the

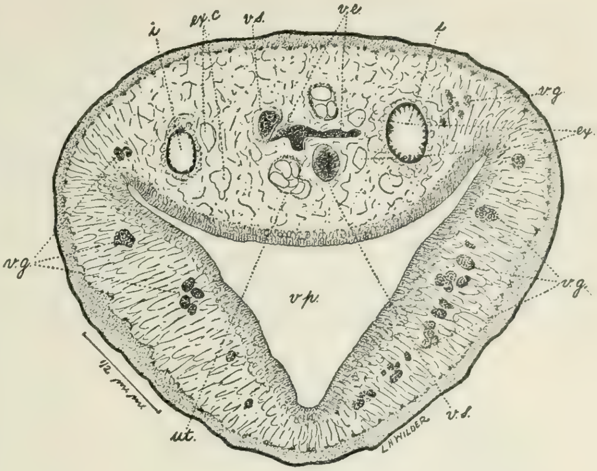

FIG. 29.

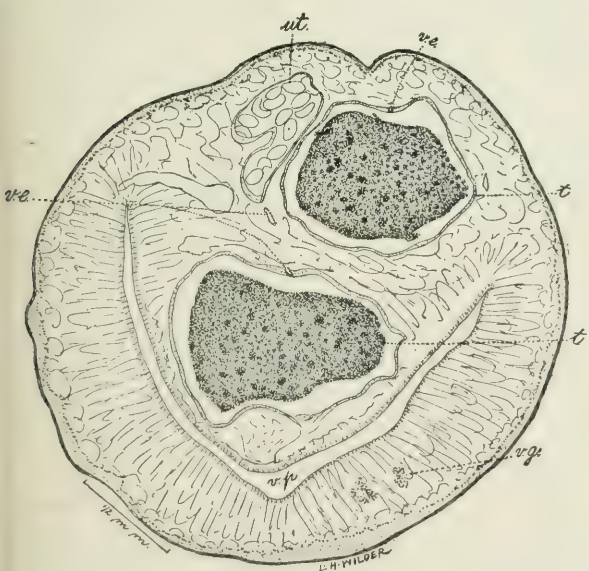

FIG. 30.

latter by the vitelloduct; these unite to form the ootype which is continued as the uterus, emerging as such from the cephalic aspect of the shell gland. From its point of emergence the uterus passes to the right, dips slightly caudad, and then ascends cephalad forming coils dorsally of the ventral and to the right of the dorsal testis. Gradually the distended coils tend toward the median line of the body and soon come to occupy the space between the vasa efferentia. When the uterus reaches the arch formed by the union of the vasa efferentia it passes beneath and ventrally of the latter and ceases to form coils. It now takes an almost direct though slightly sinuous course cephalad close to the ventral aspect of the coils formed by the vesicula and pars prostatica and finally close to the ventral aspect of the pars prostatica and ductus ejaculatorius, to open by a pore of relatively considerable size at the vertex of the genital papilla below but very close to, though apparently quite distinct from, the male genital pore. Laurer's canal leaves the oviduct at about the point where the latter turns abruptly cephalad in order to penetrate the shell gland, passes dorso-caudad, and opens by a minute pore on the dorsum about 0.60 mm. cephalad of the caudal pole of the body and about 0.36 mm. cephalad of the excretory pore.

The follicles composing the vitellogene glands (figs. 28, 30) are scattered through the ventro-lateral body segments in a manner similar to that obtaining in *F. elongatus*, and in the dorsal body segment external to the intestinal ceca. Longitudinally the gland follicles extend from a plane corresponding to the caudal end of the pars prostatica caudad to the plane of the cephalic aspect of the testes. From about the caudal extremity of each vitelline gland a duct passes medio-caudad; the two transverse ducts unite close to the ventral aspect of the shell gland at about the level of the ootype. A well-defined vitelline reservoir is not present, unless the transverse ducts which are of considerable caliber and filled with vitelline cells may be so regarded. From the point of union of the transverse ducts a duct passes off in a direction caudad close to the ventral aspect of the shell gland, and follows its contour around to its dorso-caudal aspect. Here this duct penetrates the gland to unite in its substance with the oviduct.

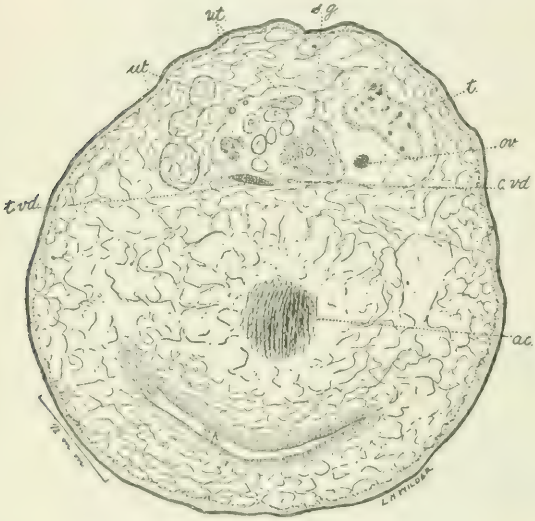

Fig. 31.

Excretory system.—The excretory vesicle lies caudo-ventrally of the ovary and shell gland and dorsally of the dome of the acetabulum. In the single specimen studied the vesicle appears for the most part as a transverse slit, distended slightly only in its caudal portion. From its caudal aspect a short duct is given off which passes dorso-caudad to open by a pore caudad of Laurer's canal. This duct is lined by a cuticle in anatomical continuation with that of the surface.

RELATION TO OTHER SPECIES.

The topography of the genital system and the termination of the intestinal ceca in the equatorial region bring this worm close to *F. elongatus* and *F. fischaderi*. The long esophagus and the roomy ventral chamber of the genital atrium, quite distinct from that in either *F. elongatus* or *F. fischaderi* appear to indicate that this is a distinct species and clearly differentiates it from both of them.

ILLUSTRATIONS.

Fig. 23.—Frontal projection. Shows oral sucker (*o. s.*), esophagus (*es.*), position of genital pore (*g. p.*), intestinal ceca (*i.*), testes (*t.*), vasa efferentia (*v. e.*), vesicula seminalis (*v. s.*), pars musculosa (*p. m.*), pars prostatica (*p. p.*), uterus (*ut.*), and acetabulum (*ac.*). *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, *h–h* planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 24.—Profile projection. Shows oral sucker (*o. s.*), esophagus (*es.*), right intestine (*i.*), testes (*t.*), right vas efferens (*v. e.*), vas deferens (*v. s.*; *p. m.*; *p. p.*), shell gland (*s. g.*), ovary (*ov.*), uterus (*ut.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), excretory pore (*ex. p.*), and acetabulum (*ac.*). *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, *h–h* planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 25.—Transverse section at *a–a* figs. 23 and 24. Shows outline of body, form of oral sucker and of its lumen (*o. s.*), perisuctorial space (*p. s. sp.*), and mesenterium-like strands (*m. b.*). Enlarged. Original.

Fig. 26.—Transverse section at *b–b* figs. 23 and 24. Shows outline of body at level of aperture of ventral pouch (*a. v. p.*) and relation of latter to beginning of the esophagus (*es.*), the perisuctorial space (*p. s. sp.*) and surface papillæ (*s. pap.*) on margin of aperture of ventral pouch. Enlarged. Original.

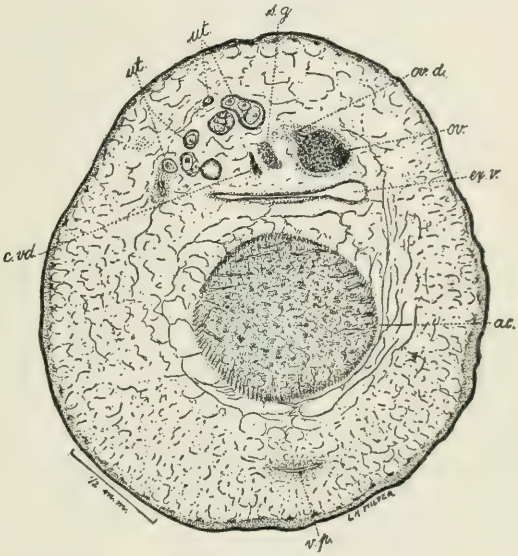

Fig. 32.

Fig. 27.—Transverse section at *c–c* figs. 23 and 24. Shows outline of body, form of ventral pouch (*v. p.*), dorsal chamber (*g. a.*), ventral chamber of genital atrium (*g. a. c.*), ductus ejaculatorius (*d. e.*), metraterm (*va.*), esophagus (*es.*), and longitudinal excretory canals (*ex. c.*). Enlarged. Original.

Fig. 28.—Transverse section at *d–d* figs. 23 and 24. Shows triangular form of body, ventral pouch (*v. p.*), intestines (*i.*), excretory canals (*ex. c.*), pars musculosa (*p. m.*) of the vas deferens, uterus (*ut.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 29.—Transverse (equatorial) section at *e–e* figs. 23 and 24. Shows form of body and of ventral pouch (*v. p.*), arch of union of vasa efferentia (*v. e.*), vesicula (*v. s.*), uterus (*ut.*), intestines (*i*), excretory canals (*ex. c.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 30.—Transverse section at *f–f* figs. 23 and 24. Shows form of body, caret-like form of ventral pouch (*v. p.*), testes (*t.*), uterus (*ut.*), origin of vasa efferentia (*v. e.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 31.—Transverse section at *g–g* figs. 23 and 24. Shows form of body, slit-like prolongation of ventral pouch (*v. p.*), superior margin of acetabulum (*ac.*), caudal aspect of dorsal testis (*t*), cephalic margin of ovary (*ov.*), shell gland (*s. g.*), transverse vitello-duct (*t. vd.*), common vitello-duct (*c. vd.*), and uterus (*ut.*). Enlarged. Original.

FIG. 32.—Transverse section at *h–h* figs. 23 and 24. Shows form of body, slit-like extremity of ventral pouch (*v. p.*), acetabulum (*ac.*), ovary (*ov.*) and origin of oviduct (*ov. d.*), caudal aspect of shell gland (*s. g.*), common vitello-duct (*c. vd.*), uterus (*ut.*), slit-like beginning of excretory vesicle (*ex. v.*). Enlarged. Original.

CARMYERIUS,[1] new genus.

GENERIC DIAGNOSIS.—*Gastrothylacinæ* (p. 16): Vas deferens and cephalic half of uterus chiefly or entirely in suctorial field. Testicular fields separate, zones nearly coincide, postcecal or intercecal; vesicula without straight portion. African and Asiatic.

TYPE SPECIES.—*C. gregarius* (Looss, 1896) [*Gastrothylax gregarius* Looss, 1896b, 5–13, 170–177, pl. 1, figs. 1–3].

This group, as at present constituted, contains 5 species which agree in certain characters, and yet are so different that they will certainly submit to further grouping as soon as additional forms become known. At present at least 4, probably 5, subgroups can be more or less clearly foreseen. For the sake of conservatism we will here recognize 5 monotypic subgenera, as follows:

A^1. Genital atrium with very large ventral chamber; testes postcecal:
  B^1. Ceca corkscrew like, rather narrow, long, end in fourth zone; testes deeply indented; transverse section of pouch triangular, apex ventrad; vitellaria fine; type *Gastrothylax synethes* Fischœder, in *Bos kerabau*, from Ceylon. New subg. *Synethes*.
  B^2. Ceca straight, broad, short, end in third zone; testes lobate, very lateral; transverse section of pouch triangular, apex dorsad; vitellaria coarse; type *Gastrothylax gregarius* Looss, in *Bos bubalus*, Egypt, also in *Bos taurus*, East Africa. New subg. *Carmyerius*.

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[1] This genus is dedicated to Miss Caroline Myer, technical clerk in the United States Bureau of Animal Industry, in recognition of the obligation which we feel that helminthologists owe to Miss Myer for her years of faithful work on the Index Catalogue of Medical and Veterinary Zoology.

A^2. Genital atrium without ventral chamber; testes post or intercecal:

B^3. Ceca straight, narrow, rather long, end in fourth zone; testes lobate, postcecal; transverse section of pouch circular; type *Gastrothylax spatiosus* Brandes, in *Bos taurus*, Arabia ....New subg. *Gastrothylacias.*

B^4. Ceca rather sinuous, narrow, long, end in fourth and fifth zones; testes intercecal; transverse section of pouch triangular, apex ventrad; type *Gastrothylax mancupatus* Fischœder, in *Bos taurus*, East Africa....New subg. *Gastrothylacides.*

B^5. Ceca swollen in caudal half, rather long, end in fourth zone; testes inter and postcecal; transverse section of pouch rather triangular, apex ventrad; type *Gastrothylax minutus* Fischœder, in *Tragelaphus scryptus*, Kamerun, also in *Antilope* sp....................................New subg. *Thylogaster.*

WELLMANIUS, new genus.

Generic diagnosis.—*Gastrothylacinæ* (p. 16): Vas deferens and cephalic half of uterus chiefly or entirely in suctorial field. Testicular fields separate, zones coincide, testes in inter, extra, and cecal areas, dorsal of ceca; vesicula seminalis consists of a caudal straight and a cephalic coiled portion.

Type species.—*Wellmanius wellmani* new species. Africa.

This new species, new genus, separates out very easily from the other forms of *Gastrothylacidæ.* Both the genus and the species are dedicated to the American student of African tropical medicine who sent us the material. The "virtual tautonomy" used here and elsewhere is intentional.

WELLMANIUS WELLMANI, new species.

[Figs. 33 to 42.]

Specific diagnosis.—*Wellmanius* (p. 51): 6.5 to 8.25 mm. long, by 3 to 4 mm. broad, more or less conical in form, straight or curved, circular in transverse section, greatest diameter near acetabulum. Surface unarmed, but bluntly pointed cephalic end bears small papillæ. Opening of ventral pouch 0.67 mm. from anterior end; pouch extends to acetabulum; its transverse section triangular (then pentagonal), with apex ventrad; shorter dorsally than ventrally, ventral end extending in acute angle, caudal wall thus not representing a transverse plane. Genital pore at about the equator of the esophagus; genital papilla may be prominent, nearly filling atrium. Acetabulum terminal, 1.7 to 2.5 mm. in diameter, its aperture tilted slightly ventrad. Oral sucker rather pyriform; esophagus, shorter than sucker, bifurcates one-fifth to one-fourth the body length into 2 tortuous wavy ceca which extend to the acetabulum. Excretory system well developed with large vesicle and with thick-walled efferent canal opening dorso-median, caudad of the opening of Laurer's canal.

Male organs: Testes lobate, lateral, in same transverse zone, but their fields are separated by dome of acetabulum and excretory vesicle; vas deferens median; vesicula seminalis, pars musculosa, pars prostatica, and ductus ejaculatorius well developed.

Female organs: Ovary usually left, shell gland right of median line, between cephalic end of testes; uterus coiled; metraterm straight; Laurer's canal opens median, cephalad of excretory pore; vitellaria extend from slightly caudad of intestinal fork to near termination of ventral pouch; at first (cephalad) the follicles are small, sparse, and scattered, then larger and more numerous, extending ventrad of intestinal ceca and ventral pouch.

Eggs: 112.5 by 60μ, oval, operculated.

Habitat.—Lesser Reed Bok (*Cervicapra bohor*), Benguella (type locality), Africa.

Type.—U.S.P.H.&M.H.S. 9831, alcoholic material and sections.

SOURCE OF MATERIAL.—This material was sent to us by the American Society of Tropical Medicine. It was collected by Dr. F. C. Wellman in Benguella, West Africa, from the stomach of the Lesser Reed Bok (*Cervicapra bohor*). The sending contains a piece of stomach showing how these trematodes attach themselves to the mucosa.

FIG. 33.

EXTERNAL CHARACTERS.

SIZE.—The specimens vary in size from 6.5 to 8.25 mm. in length by 3 to 4 mm. in breadth.

COLOR.—Alcohol specimens are of a buff color.

FORM.—The specimens differ somewhat one from another, but all are more or less conical in form, with the longitudinal axis either straight or more or less curved with the convexity dorsad. The cephalic extremity is rather bluntly pointed and bears the terminal mouth which may be directed slightly dorsad or slightly ventrad. The body is more or less circular in cross section and its greatest diameter is more or less near the caudal extremity which bears a large terminal acetabulum, the terminal circular orifice of which, in some of the specimens, is also tilted slightly ventrad. In some of the specimens the body is somewhat constricted transversely at or slightly caudad of the equator (figs. 33, 34).

SURFACE.—The surface cuticle is devoid of spines or hooks, but is marked by fine transverse sulci and at the oral extremity it is sparsely beset by bluntly rounded papillæ. It is marked at one point, a little (0.675 mm. in one series of sections) below the oral margin, by a small transverse slit, which is the aperture of the ventral pouch and indicates the position of the venter of the animal. Immediately above this slit the surface over a circumscribed area is slightly depressed (see figs. 33, 34).

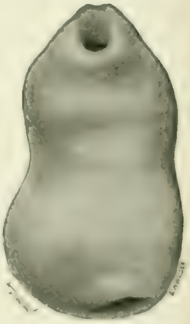


FIG. 34.

Ventral pouch.—The ventral pouch extends from its aperture, which is situated a little (0.675 mm. in a sagittal section) below the level of the oral margin to a little above the level of the upper margin of the acetabulum. In transverse sections the form and dimensions of its lumen vary greatly at different levels and to a considerable extent in different specimens. The aperture lies in a slight depression of the venter. It is crescentic and more or less slit like in form (fig. 37), measuring 0.39 mm. from horn to horn in one of the sectioned specimens. A little below the level of the genital pore, which is on he dorsal wall of the pouch, the outline in transverse sections is ound to have become changed from the crescentic slit to nearly a triangular form with the apex ventrad (fig. 38).

In its further course caudad the triangular lumen of the pouch radually shifts toward the axial region of the body which it then omes to occupy. Coincident with this shifting of position there is further change of outline, the triangular form becoming more or ess pentagonal but with the apex still directed ventrad (fig. 39). At first the caliber of the lumen of the pouch increases, but as the fundus of the pouch is approached, its ventro-dorsal diameter becomes rapidly contracted because the fundus instead of occupying a horizontal (transverse) plane occupies a plane which is directed obliquely caudo-ventrad, the dorsal wall of the pouch being shorter than the ventral; in consequence of this, the outline of the pouch in sagittal section is somewhat that of a triangle with its apex at the entrance of the pouch (fig. 35). The lining of the pouch is a cuticle in anatomical continuation with that of the surface and appears to be in irregular longitudinal and transverse folds, producing an appearance somewhat suggestive of papillæ in sections. In the specimens sectioned, the pouch contained some granular matter and a few eggs.

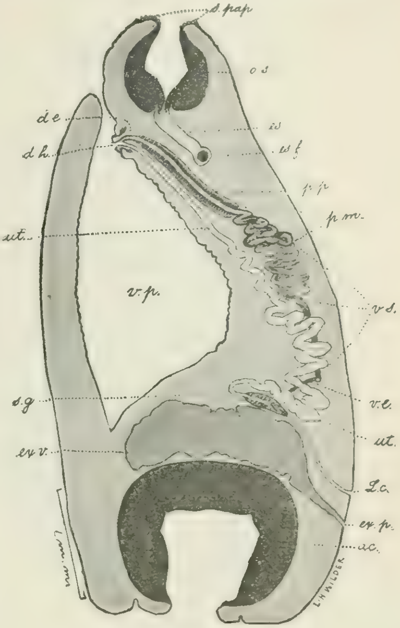


Fig. 35.

Genital pore.—On the dorsal wall of the pouch a little (0.375 mm. in one sagittal section) below its aperture and in about the equator of the esophagus is the genital pore. This pore is at the vertex of a somewhat flattened hemispherical bulging. In a sagittal section of one specimen this bulging measured 0.40 mm. in its longitudinal diameter. The genital pore leads into a chamber which is almost completely filled by a papilla (genital). In one sectioned specimen a part of this papilla protruded into the ventral pouch through the pore, its margin closely embracing and somewhat constricting it (fig. 35).

Acetabulum.—This muscular organ of adhesion occupies the caudal extremity and its aperture, which is more or less circular, is in the axial line with a slight tilt ventrad in some of the specimens. The diameter of the acetabulum varied from 1.7 to 2.5 mm. in the specimens in which it was measured. The ventro-dorsal diameter of the aperture in one specimen measured 0.40 mm.

INTERNAL ANATOMY.

Digestive tract.—The bluntly pointed cephalic extremity is pierced by the more or less circular mouth which leads directly into a very muscular, somewhat pyriform, oral sucker. In cross (transverse) section the outline of the sucker varies in different specimens from an ellipse to a circle. The oral pole of the sucker extends to the surface where it forms a ring around the oral aperture, being marked off from the general body surface by a narrow more or less deep encircling groove (fig. 40). Inclosing the sucker is a space which is traversed, dorsally and ventrally, by dorso-ventral strands. At the esophageal end the sucker is attached to the body parenchyma so that the organ is anchored at both its poles.

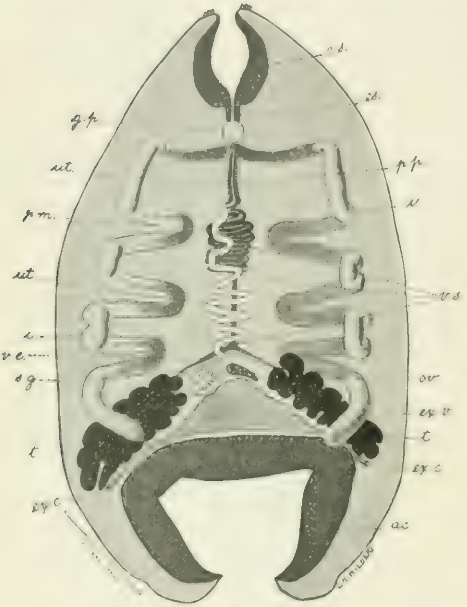


Fig. 36.

The lumen of the sucker, when studied in transverse sections, is circular in outline at its oral end, becoming somewhat spindle shaped in the equatorial region; beyond this region the lumen at first becomes dorso-ventrally contracted, forming a transverse slit; this eventually becomes reduced to a small circular orifice at the entrance into the esophagus. It is lined by a thin cuticle-like layer, anatomically continuous with that of the surface, and it is beset by small more or less conical papillæ; these papillæ are sparsely scattered in the region of the mouth but numerous and closely aggregated in the remaining portion (figs. 37, 40). From its origin at the base of the sucker the esophagus passes caudo-dorsad; at a point a little less than one-fifth to one-fourth the body length from the oral margin it forks into two intestinal ceca. The esophagus is somewhat shorter than the sucker.

From their point of origin the intestinal ceca, without giving off any branches, pass at first latero-caudad, then having reached the dorso-lateral region of the body they take a dorso-ventrally wavy course caudad to terminate ventrad of the corresponding testis at or a little above the level of the upper margin of the acetabulum (fig. 36, 41).

The lumen of the esophagus is lined by a thick layer of cuticle. At the point of origin of the intestinal ceca the cuticular lining is replaced by a nucleated cell layer which extends throughout the remaining length of the intestine.

The intestinal ceca do not appear to be exactly of equal length, nor is the level at which they terminate absolutely fixed. They may terminate at the level indicated or somewhat above (cephalad of) that point.

GENITAL SYSTEM.—*Male organs:* The two testes are situated in the same transverse zone near the caudal extremity of the worm at or just cephalad of the level of the anterior (cephalic) portion of the acetabulum, and separated in the former case by the dome of the acetabulum, ventrally, and the excretory vesicle, dorsally, and in the latter case by the excretory vesicle alone (figs. 36, 41, 42). The testes are somewhat compressed laterally and their margins are deeply indented, the indentations marking off lobes of different dimensions. Cephalo-ventrad each is in relation with the corresponding cecum. From the dorsal aspect of each gland arises a vas efferens; these two ducts take a course at first dorsad and slightly cephalad, then toward the median line, and unite at a point a little above the level of the upper margin of the testes, to form the vas deferens (fig. 36). The vas deferens is clearly divisible into a vesicula, musculosa, prostatica, and ejaculatorius. The vesicula may be described as consisting of two portions; the first pursues quite a straight course cephalad, close under the dorsum, about in the median line, close to the dorsal aspect of the uterus, and with rather a thick wall proportionate to its empty contracted lumen. The second portion of the vesicula forms numerous compact coils, its walls are thinner, and the caliber of its lumen is increased. Farther cephalad the wall changes in structure, becoming quite muscular (pars musculosa), while the duct still forms compact coils; the cephalic end of the pars musculosa turns abruptly ventrad, the duct becomes surrounded by numerous cells and loses its muscular layer, turns sharply cephalad, and proceeds as the pars prostatica toward the genital pore. It passes ventro-cephalad of the intestinal arch, runs dorsally of the metraterm, ventrally of the esophagus, decreases gradually in diam-

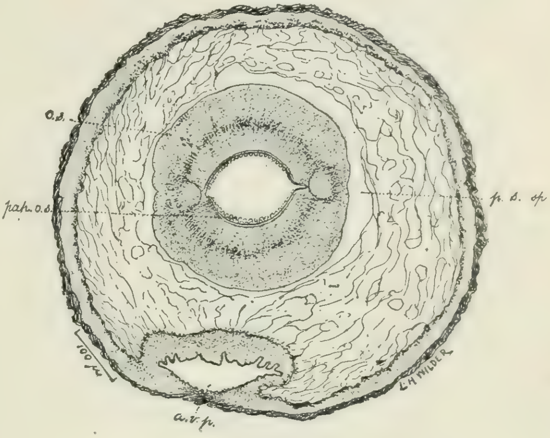


FIG. 37.

eter, and loses its prostatic cells; it now continues as the ductus ejaculatorius. The latter enters the genital papilla, and unites with the metraterm to form the short ductus hermaphroditicus, which opens at the vertex of the papilla as the porus hermaphroditicus (fig. 35).

Female organs.—The ovary and the shell gland are in the caudal portion of the body, but further than this considerable variation was observed in their position and their relation to each other and to the excretory vesicle. This was thought to be dependent in part on the degree of distention of the excretory vesicle. The ovary lies immediately to the right or to the left of the median sagittal plane, cephalo-dorsad or dorsad of the excretory vesicle and somewhat above the level of the superior margin of the acetabulum. In 3 of 4 sectioned specimens it was to the left of the median plane; in the fourth it was to the right. In 3 of the 4 specimens the ovary was close to the dorsal portion of the cephalic aspect of the excretory vesicle (fig. 35), rather than dorsally of the body of the vesicle. With respect to its relation to the acetabulum, it was found that while in 3 of the 4 specimens the ovary was cephalad of the dome of the acetabulum, with the excretory vesicle interposed (fig. 36), in the fourth series of sections the ovary was distinctly below (caudad of) the transverse plane of the superior margin of the acetabulum. In all cases it was distinctly nearer the dorsum than the venter. The shell gland lies close to the right or to the left and more or less cephalad of the ovary, in one instance, cephalo-ventrad of the ovary. Both organs vary in form in the different specimens; for the most part they are somewhat flattened, oblong bodies, but may appear pyriform or subglobular. The ovary is somewhat the larger of the two. From the aspect of the ovary nearest the shell gland the oviduct arises and passes to the shell gland, which it penetrates. Just before entering the latter it gives off Laurer's canal. In the shell gland it is at once joined by the vitello-duct; the common duct so formed then dilates some-

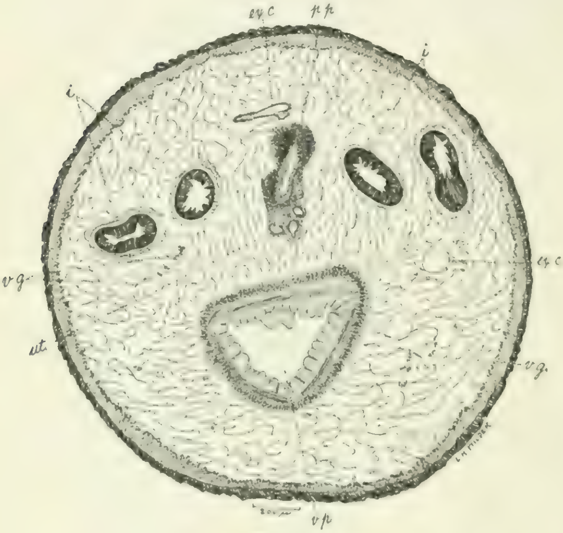


FIG. 38.

what to form the ootype and beyond this point the canal is continued as the uterus, which emerges from the shell gland at the ventro-cephalic aspect. The uterus then winds its way dorsad over the shell gland; a coil of it forms a loop which is tucked in between the dorsum, on the one hand, and the shell gland and the ovary on the other, before beginning its course cephalad. In the first part of its course cephalad it is included between the two vasa efferentia, but passes ventrad beneath the arch formed by their union to gain the ventral aspect of the vas deferens. It maintains this ventral relation to the vas deferens and its terminal portion, pars prostatica, etc., in the remainder of its course to the genital papilla. As already mentioned, it opens near the vertex of the latter into a short duct (ductus hermaphroditicus) common to it and the ductus ejaculatorius.

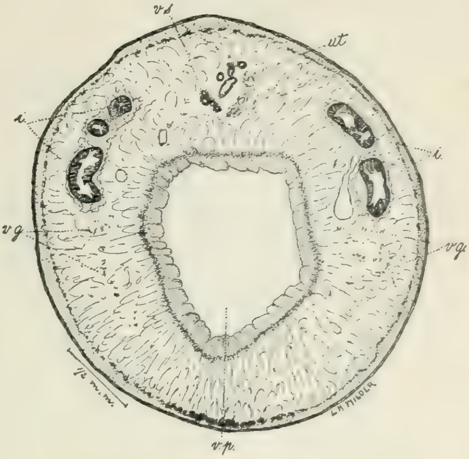


Fig. 39.

Numerous eggs were observed filling the loops here and there and, near the shell gland, some of the loops contained masses of spermatozoa.

Laurer's canal passes from its point of origin dorso-caudad to open in the median line slightly below the upper margin of the acetabulum and a little above the excretory pore. The canal is long and slender; it is lined by a thick cuticle in anatomical continuation with the cuticle of the surface, and its lumen may contain cells resembling those in the vitelloducts. Receptaculum seminis is wanting.

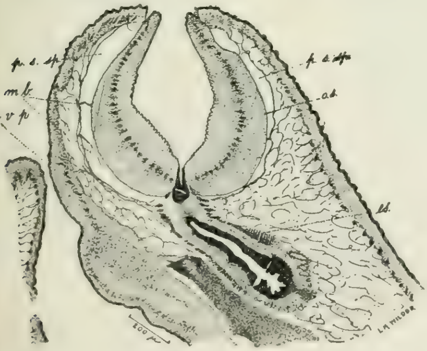


Fig. 40.

The vitellaria begin in a transverse plane somewhat caudad of the esophageal bifurcation. At first there are a few small scattered follicles ventrad of the intestinal ceca, but gradually follicles appear which are larger and somewhat more numerous and scattered close around and ventrally of the intestinal ceca and ventral pouch. They extend caudad to a plane slightly above (cephalad of) the termination of the ventral pouch; at about this point and ventro-centrad of the intestinal ceca, the vitello-ducts arise, and each passes medio-dorso-caudad or caudad (between the testes); the two ducts unite about in the median line immediately cephalad of the origin of the excretory vesicle. The common duct thus formed passes dorsad toward the shell gland and pierces the caudal or the right (? or in some specimens left) lateral aspect of this gland to join the oviduct just before the latter dilates into the ootype.

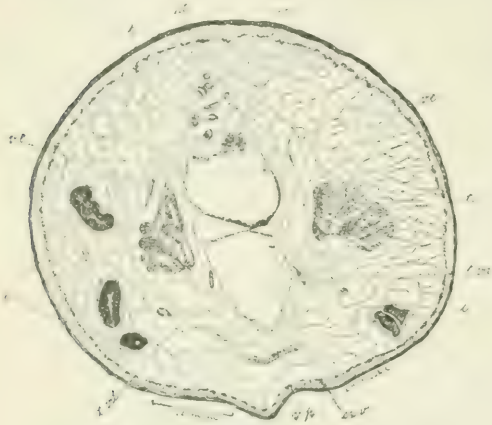


FIG. 41.

Eggs.—The eggs are oval in outline and provided with a small operculum at one end. We found some difficulty in obtaining them in a condition satisfactory for measurement. One egg measured 112.5 by 60μ.

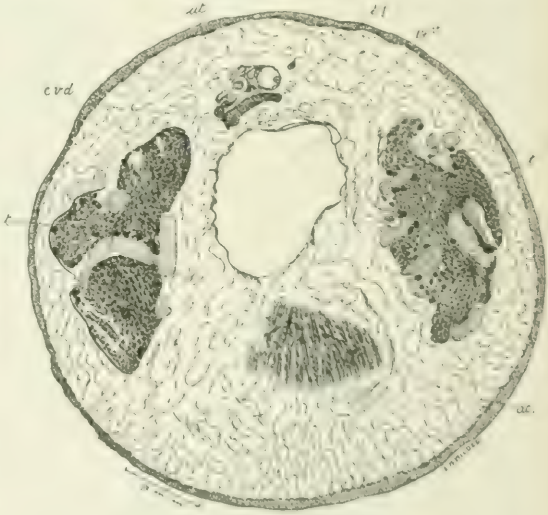


FIG. 42.

EXCRETORY SYSTEM. The excretory vesicle, when distended, is of considerable size. It lies immediately above the acetabulum and between the two testes. Its ventro-dorsal diameter is about equal to the corresponding diameter of the acetabulum. It receives on either side, at about its ventro-lateral aspect, a large excretory canal, which may be observed to wind its way from the cephalic portion of the body caudad on the ventro-lateral aspect of the corresponding testis, then beneath the latter to its destination. From the dorso-caudal aspect of the vesicle a thick-walled canal arises, which after a short course caudo-dorsad opens on the dorsal surface at the excretory pore a little below the opening of Laurer's canal (fig. 35).

ILLUSTRATIONS.

FIG. 33.—External appearance, ventral view. Enlarged. Original.

FIG. 34.—External appearance, ventral view. Enlarged. Original.

FIG. 35.—Diagrammatic sagittal section, showing internal anatomy: *ac.*, acetabulum; *d. e.*, ductus ejaculatorius; *d. h.*, ductus hermaphroditicus; *es.*, esophagus; *es. f.*, esophageal fork; *ex. p.*, excretory pore; *ex. v.*, excretory vesicle; *i.*, intestinal ceca; *L. c.*, Laurer's canal; *o. s.*, oral sucker; *p. m.*, pars musculosa; *p. p.*, pars prostatica; *s. g.*, shell gland; *s. pap.*, surface papillæ; *v. e.*, vasa efferentia; *v. p.*, ventral pouch; *v. s.*, vesicula seminalis; *ut.*, uterus. Enlarged. Original.

FIG. 36.—Diagrammatic frontal section shows internal anatomy: *g. p.*, position of genital pore; *ex. c.*, excretory canals; *ov.*, ovary; *t.*, testes. For other abbreviations see fig. 35. Enlarged. Original.

FIG. 37.—Transverse section at level of aperture of ventral pouch. Shows form of body, aperture of ventral pouch (*a. v. p.*), oral sucker (*o. s.*), papillæ of suctorial lumen (*pap. o. s.*), and the perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 38.—Transverse section slightly caudad of level of genital pore. Shows form of body, triangular outline of ventral pouch (*v. p.*) at this level the intestinal ceca (*i.*) and their relation to the ventral pouch, to pars prostatica (*p. p.*) and uterus (*ut.*), excretory canals (*ex. c.*) and vitellaria (*v. g.*). Enlarged. Original.

FIG. 39.—Transverse section at about the junction of the second with the equatorial fifth of the body length. Shows form of body, irregularly pentagonal form of ventral pouch (*v. p.*), intestinal ceca (*i*), vesicula seminalis (*v. s.*), uterus (*ut.*), and vitellaria (*v. g.*) Enlarged. Original.

FIG. 40.—Sagittal section showing oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), mesenterium-like strands (*m. b.*), esophagus (*es.*), and aperture of ventral pouch (*ap. v. p.*). Enlarged. Original.

FIG. 41.—Transverse section immediately cephalad of superior margin of acetabulum shows the laterally placed testes (*t.*) separated by the excretory vesicle (*ex. v.*) and the position and relation of the (caudal portion of the) ovary (*ov.*), uterus (*ut.*), vasa efferentia (*v. e.*), intestinal ceca (*i.*), transverse vitello-ducts (*t. vd.*) and caudal terminal portion of ventral pouch (*v. p.*). Enlarged. Original.

FIG. 42.—Transverse section immediately below superior margin of acetabulum. Shows the laterally placed testes (*t.*) separated by

the acetabulum (*ac.*) and excretory vesicle (*ex. v.*). Shows also the shell gland (*s. g.*), uterus (*ut.*), and common vitello-duct (*c. vd.*). Enlarged. Original.

Family PARAMPHISTOMIDÆ Fischœder, 1901, restricted.

FAMILY DIAGNOSIS. *Paramphistomoidea* (p. 15): Ventral pouch absent.
TYPE GENUS.—*Paramphistomum* Fischœder, 1901.

The family *Paramphistomidæ* Fischœder, 1901, is here restricted to the forms without a ventral pouch. Our work is based primarily upon the amphistomes of mammals, but it seems not entirely excluded that further study of the amphistomes of other vertebrates may result in a further restriction of the family to forms in which the oral sucker is without an evagination.

Fischœder (1901a), in writing upon species found in mammals, recognized 2 subfamilies for *Paramphistomidæ*, namely, *Paramphistominæ* and *Cladorchiinæ*. Cohn (1904), in dealing with species found in amphibians and reptiles, recognized the subfamily *Diplodiscinæ* for three genera, but he did not compare his forms with the forms discussed by Fischœder. Daday (1907) later described forms from fish, classifying some of them near some of the forms described by Fischœder, others in *Diplodiscus*, but he did not enter into a discussion of the classification of the subfamilies. Disregarding the new genera proposed in the present paper, the classification stands at present as follows:

- *Paramphistomidæ* Fischœder, 1901.
  - *Paramphistominæ* Fischœder, 1901.
    - *Paramphistomum* Fischœder, 1901.
    - *Stephanopharynx* Fischœder, 1901.
    - *Gastrothylax* Poirier, 1883.
  - *Cladorchiinæ* Fischœder, 1901.
    - *Cladorchis* Fischœder, 1901.
      - Subg. *Cladorchis* Fischœder, 1901.
      - Subg. *Taxorchis* Fischœder, 1901.
      - Subg. *Stichorchis* Fischœder, 1901.
    - *Microrchis* Daday, 1907.
    - *Pseudocladorchis* Daday, 1907.
    - *Pseudodiscus* Sonsino, 1895.
    - *Gastrodiscus* Leuckart, 1877.
    - *Homalogaster* Poirier, 1883.
  - *Diplodiscinæ* Cohn, 1904.
    - *Diplodiscus* Diesing, 1836.
    - *Catadiscus* Cohn, 1904.
    - *Opisthodiscus* Cohn, 1904.
  - Subfamily not given.
    - *Balanorchis* Fischœder, 1901.

When we consider the status of this group prior to 1901, the great advance in our knowledge which we owe to Fischœder must be immediately recognized, and this advance has been further supported, especially by Cohn and Daday. That the classification as it now stands is not final lies in the nature of things; and on account of the fact that definite data are lacking in the case of many species it must be expected that in the future rather radical changes may perhaps be necessary. In the immediate future, changes are more or less experimental, though they may appear justified.

Gastrothylax should, we believe, be placed in a separate family (see p. 16).

Stephanopharynx presents such a striking character in its circular evagination of the oral sucker that it seems wise to separate it entirely from *Paramphistominæ;* but if left in this subfamily, it should at least be placed in a tribe distinct from *Paramphistomum.*

Gastrodiscus and *Homalogaster* are so different from the other members of *Cladorchiinæ* that they should either be placed in a distinct subfamily or in a distinct family.

Diplodiscinæ will probably be justified as a distinct subfamily, or at least as a distinct tribe, but further data are desirable in regard to its genera.

Balanorchis is placed with difficulty. It probably represents a distinct subfamily; if classified in *Cladorchiinæ,* it should represent a distinct tribe.

It is exceedingly difficult at present to draft a key representing the natural relations of members of this family, but the following key, admittedly more or less artificial, will be found of use in tracing the genera. This key is based primarily upon the type species:

Key to the Known and New Genera of Paramphistomidae s. str.

A^1. Oral sucker without evagination [this probably represents a distinct subfamily *Paramphistominæ,* p. 62]; cirrus pouch absent; body not divided; ovary not pretesticular [eventually these may be tribal or subtribal characters]:

  B^1. Genital sucker absent; type *cervi*......................*Paramphistomum,* p. 73. [For subgenera, see p. 74.]

  B^2. Genital sucker present; type *cotylophorum*..................*Cotylophoron,* p. 63.

A^2. Oral sucker with evagination:

  B^3. Evagination circular [this will probably prove to be of supergeneric value, indicating a subtribe or tribe, probably a subfamily, *Stephanopharynginæ*]; genital sucker and cirrus pouch absent, body not divided [these characters will probably prove to be supergeneric]; type *compactus.*
  Stephanopharynx, p. 168.

  B^4. Evagination paired [if of subfamily value, this unites *Cladorchiinæ* and *Diplodiscinæ* in one subfamily; later investigations may show that these groups can be separated on the excretory system, cf. *Diplodiscus*]:

    C^1. Genital sucker present; testes clearly branched:

      D^1. Vitellaria entirely posttesticular; testicular zones coincide, preequatorial, widely separated from acetabulum, fields separate; uterus of unusual course, first runs near dorsum cephalad to testes, then diagonally caudad near venter, then cephalad near venter to pore; type *schistocotyle.*
      Taxorchis, p. 170

      D^2. Vitellaria not entirely posttesticular; testes in equatorial third; uterus not as in *Taxorchis;* type *pyriformis*..................*Cladorchis,* p. 169.

C^2. Genital sucker absent; testes lobate or lobulate:
  D^3. Cirrus pouch absent:
    E^1. Each oral evagination single (only pouch present), type *watsoni*. *Watsonius*, p. 212.
    E^2. Each oral evagination double (pouch and bulb present), type *stanleyii*. *Pseudodiscus*, p. 170.
  D^4. Cirrus pouch present [perhaps in part a special subfamily, see *Diplodiscinæ*, p. 247]:
    E^3. Acetabulum divided into two parts by constriction; one testis present; esophagus with muscular swelling at bifurcation; type *dolichocotyle*. *Catadiscus*, p. 248.
    E^4. Acetabulum with central projecting sucker; two testes present; esophagus without muscular swelling; type *diplodiscoides*. *Opisthodiscus*, p. 248.
    E^5. Acetabulum cavity with prominent papillæ; excretory system does not enter acetabulum; testes preovarial, at least not postovarial; evaginations of sucker well developed, at least not confined to wall; esophagus without pronounced muscular thickening; testicular zones coincide; excretory pore vesicular, type *papillatus*............*Pfenderius*, p. 232.
    E^6. Acetabulum otherwise:
      F^1. Excretory system branches radially in acetabulum [perhaps special subfamily *Diplodiscinæ*, with *Diplodiscus*, *Catadiscus*, *Opisthodiscus*]; testes coalesce in adult; esophagus with muscular swelling at bifurcation; type *subclaratus*.........................*Diplodiscus*, p. 248.
      F^2. Excretory system does not appear to enter acetabulum:
        G^1. Testes postovarial, portuterine, postequatorial; type *anastrophus* *Balanorchis*, p. 247.
        G^2. Testes preovarial, at least not postovarial:
          H^1. Evaginations of sucker not well developed, confined to wall of sucker; sucker with two phincters; type *cylindricus*. *Pseudocladorchis*, p. 232.
          H^2. Evaginations of sucker well developed, at least not confined to wall; sucker with one sphincter; esophagus with muscular thickening; testicular zones separate; excretory pore prevesicular:
            I^1. Testes not lobate; excretory pore equatorial; type *megacotyle*. *Microrchis*, p. 246.
            I^2. Testes 4-lobate; excretory pore postequatorial, posttesticular, with powerful sphincter; type *fabaceus*....*Chiorchis*, p. 246.

Subfamily PARAMPHISTOMINÆ, restricted.

SUBFAMILY DIAGNOSIS.—*Paramphistomida* (p. 60): Oral sucker without evagination.
TYPE GENUS.—*Paramphistomum*.

There seems every indication that the group here defined will represent a subfamily.

Additional characters for the two known genera are: Cirrus pouch absent, ovary not pretesticular, excretory system does not enter acetabulum.

As additional genera become known, these characters may result in dividing the subfamily into tribes and subtribes.

The two known genera may easily be distinguished as follows:

Genital sucker absent; type *cervi*. ... .*Paramphistomum*, p. 73.
Genital sucker present; type *cotylophorum*.....................*Cotylophoron*, p. 63.

COTYLOPHORON, new genus.

GENERIC DIAGNOSIS.—*Paramphistominæ* (p. 62): Esophagus with or without muscular thickening; ceca long, wavy, end in acetabular zone. Acetabulum of moderate size, terminal, tilts ventrad. Excretory vesicle and canal directed cephalad; excretory pore prevesicular. *Genital sucker present*, genital papilla present, ventral chamber of genital atrium absent.

Male organs: Testes smaller than acetabulum, lobate, immediately preacetabular, zones overlap slightly, fields nearly coincide, crossing median line.

Female organs: Ovary ventral of excretory vesicle; Laurer's canal crosses excretory vesicle; its pore opens caudad and laterad of excretory pore, in acetabular and vesicular zones.

TYPE SPECIES.—*Cotylophoron cotylophorum* (Fischœder, 1901).

Thus far, two easily separated species may be classified in this genus. They are distinguished as follows:

Esophageal muscular thickening present; genital pore bifurcal; body 5 to 8 mm. long; type hosts: *Bos taurus*, Togo; *Bos taurus indicus*, German East Africa. *C. cotylophorum*, p. 63.

Esophageal muscular thickening absent; genital pore postbifurcal; body, 4.3 to 5.5 mm. long; type host: *Ovis aries*, India........................*C. indicum*, p. 63.

Species COTYLOPHORON COTYLOPHORUM (Fischœder, 1901) Stiles & Goldberger, 1910.

1901: *Paramphistomum cotylophorum* Fischœder, 1901a, 370 (stomach of *Bos taurus*, Togo; *Bos zebu*, German East Africa).

1909: *Cotylophoron cotylophorum* (Fischœder, 1901) Stiles & Goldberger, 1910a, 63. For full bibliography, see Stiles & Hassall, 1908, Index Catalogue, etc., Trematoda.

COTYLOPHORON INDICUM, new species.

[Figs. 43 to 52.]

SPECIFIC DIAGNOSIS.—*Cotylophoron* (p. 63): Body 4.3 to 5. 5 mm. long by 1.5 to 2 mm. broad; color (in glycerine alcohol) faint brown tint; slender, conical in form, greatest breadth near caudal extremity; tapers to bluntly pointed oral extremity; longitudinal axis curved, concavity ventrad; dorsum convex longitudinally and transversely, venter concave longitudinally, convex transversely; lateral margins slightly excurvate longitudinally, convex dorso-ventrally; transverse section elliptical. Surface without spines or papillæ except possibly at oral aperture which may (?) bear minute papillæ. Genital pore distinctly postbifurcal, on vertex of slight but not sharply defined bulging about one-third of body length from oral pole, and surrounded by genital sucker. Acetabulum caudal, sunken so that apparent aperture is formed by body, margin not projecting, 1.14 mm. in dorso-ventral diameter, aperture tilted somewhat ventrad, 0.6 mm. in diameter, muscular dome 0.24 mm. thick. Mouth at blunt cephalic extremity; oral sucker pyriform in sagittal section, its lumen papillate, narrow dorso-ventrally, rather broad transversely; maximum measurements of sucker 0.52 mm. long, 0.42 mm. in dorso-ventral and 0.6 mm. in transverse diameter; perisuctorial space roomy; esophagus about two-thirds as long as sucker and curved slightly ventrad; ceca arise from dorso-lateral aspects of end of esophagus, slightly less than one-fourth the body length from oral extremity, at first forming with each other a very acute angle they approach the lateral margins of body, then extend caudad in well-marked dorso-ventrally wavy course to terminate about ⅚ of body length from mouth, postovarial, in acetabular zone, the left cecum ending slightly caudad of the right; ceca of relatively considerable caliber. Excretory pore dorso-median about at junction of equatorial with caudal third of body, about 0.16 mm. cephalad of acetabulum, cephalad of caudal margin of caudal testis and about 0.56 mm. cephalad of pore of Laurer's canal; excretory canal short, thick walled, runs caudo-ventrad to well-developed elongate, slightly bag-shaped vesicle, the fundus of which is close to dome of acetabulum, intercecal, at end of ceca.

Male organs: Testes large, but somewhat smaller than acetabulum, irregularly globular, lobate, in median line, cephalad of acetabulum, intercecal, fields nearly coincide, zones overlap; union of vasa efferentia slightly cephalad of cephalic testis; vas deferens coiled; its vesicula seminalis much coiled, dilated; pars musculosa not coiled, narrow; pars prostatica about as long as musculosa, directed ventrad; ductus ejaculatorius short, unites with metraterm to form ductus hermaphroditicus, which is 195μ long and opens at porus hermaphroditicus on vertex of cylindrical genital papilla, which is 105μ long, 75μ in diameter; the latter almost fills a chamber 105μ deep, 105μ in diameter; this in turn is surrounded by a hemispherical, sharply defined, muscular genital sucker.

Female organs: Ovary and shell gland in testicular to posttesticular zones, intercecal, sinistral, close to acetabulum in cephalic portion of acetabular zone, shell gland in ovarian and postovarian zones; vitellaria with sparse and scattered, but well-developed follicles, chiefly extra-cecal, extending throughout cecal zone and slightly postcecal; a few follicles enter also the cecal and intercecal areas; uterus extends from ventral pole of shell gland, runs cephalo-ventrad, forming coils, turns dorsad, skirting the left lateral aspect of the caudal testis, turns cephalad dorsally of this testis, passes dorsally of both testes, ventrad over cephalic margin of cephalic testis, under arch of vasa efferentia, cephalad again ventrally of vas deferens to penetrate genital sucker caudad of penetration of male duct, and unites with latter in wall of genital sucker to form the ductus hermaphroditicus; Laurer's canal passes from oviduct in a curve (convexity cephalad) dorsally, crossing on left aspect of excretory vesicle to left of dorso-median line and opens dorsally of excretory vesicle, about 0.56 mm. caudad of excretory pore.

Eggs: Not observed.

Type.—U.S.N.M. 5781.

Habitat.—In (? organ of) sheep, *Ovis aries*, India.

Source of material.—Six specimens, in bottle of the Hassall Collection bearing the Smithsonian number 5781, were labeled: "*Amphistoma conicum;*" host, *Ovies aries;* locality, India; presented May 19, 1886.

EXTERNAL CHARACTERS.

Size.—The specimens (in glycerine alcohol) varied in length from 4.3 to 5.5 mm. and in width from 1.5 to 2 mm.

Color.—The specimens in glycerine alcohol are of a faint brown tint.

Form.—The worms are of a slender conical form, broadest near the caudal extremity and tapering to a bluntly pointed attenuated oral extremity. The longitudinal axis is more or less curved, with the concavity directed ventrad. The dorsum is convex longitudinally and transversely, the venter is concave in the longitudinal direction, but convex transversely, its convexity being less marked, however, than the corresponding convexity of the dorsum. The lateral margins are slightly excurvate in the longitudinal direction and convex dorso-ventrally. In transverse section the body of the worm is elliptical in outline, with a somewhat greater transverse than dorso-ventral diameter.

SURFACE.—The general cuticular surface is smooth; that is, unprovided with spines or papillæ. Around the oral aperture some minute papillæ may be present, but this could not be determined with certainty.

Genital pore.—In the median sagittal line of the venter, about one-third of the worm's length from the oral extremity, there is the genital pore. It is situated at the vertex of a slight but not sharply defined ventral bulging.

Acetabulum.—This is in the caudal extremity, with a terminal though a more or less ventrally tilted aperture. The rim is sunken or more or less retracted beneath the inclosing body surface (fig. 45), so that the (apparent) aperture is formed by the inclosing parenchyma. Measured from a projection of one sectioned specimen (which measured 3.9 mm. in length), the maximum dorso-ventral diameter of the acetabulum was 1.14 mm. and of its aperture 0.60 mm., with a thickness of dome of about 0.24 mm.

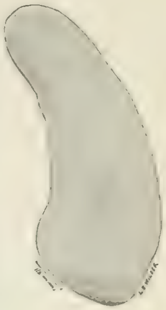


FIG. 43.

INTERNAL ANATOMY.

The following description of the internal anatomy is based on a series of transverse sections of one specimen (5781a) which measured 3.90 mm. in length, 1.66 mm. in greatest transverse and 1.42 mm. in greatest dorso-ventral diameter as measured from projections made from the sections.

DIGESTIVE TRACT.—The mouth, which pierces the blunted cephalic extremity, leads directly into the oral sucker. The latter is muscular and of a pyriform outline as viewed in median sagittal section. Its maximum dimensions as measured in projection of a series of transverse sections are: Length, 0.52 mm.; dorso-ventral diameter, 0.42 mm.; transverse diameter, 0.60 mm. Its attenuated pole presents the aperture of the mouth and is marked off from the oral surface by a narrow encircling groove (fig. 45). Its broad blunt base gives origin to the esophagus. A roomy space incloses the body of the sucker (fig. 47). The lumen of the sucker is a dorso-ventrally narrow but transversely a rather broad space; it is lined with a cuticle which is beset with short conical papillæ.

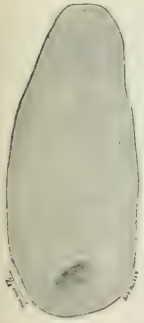
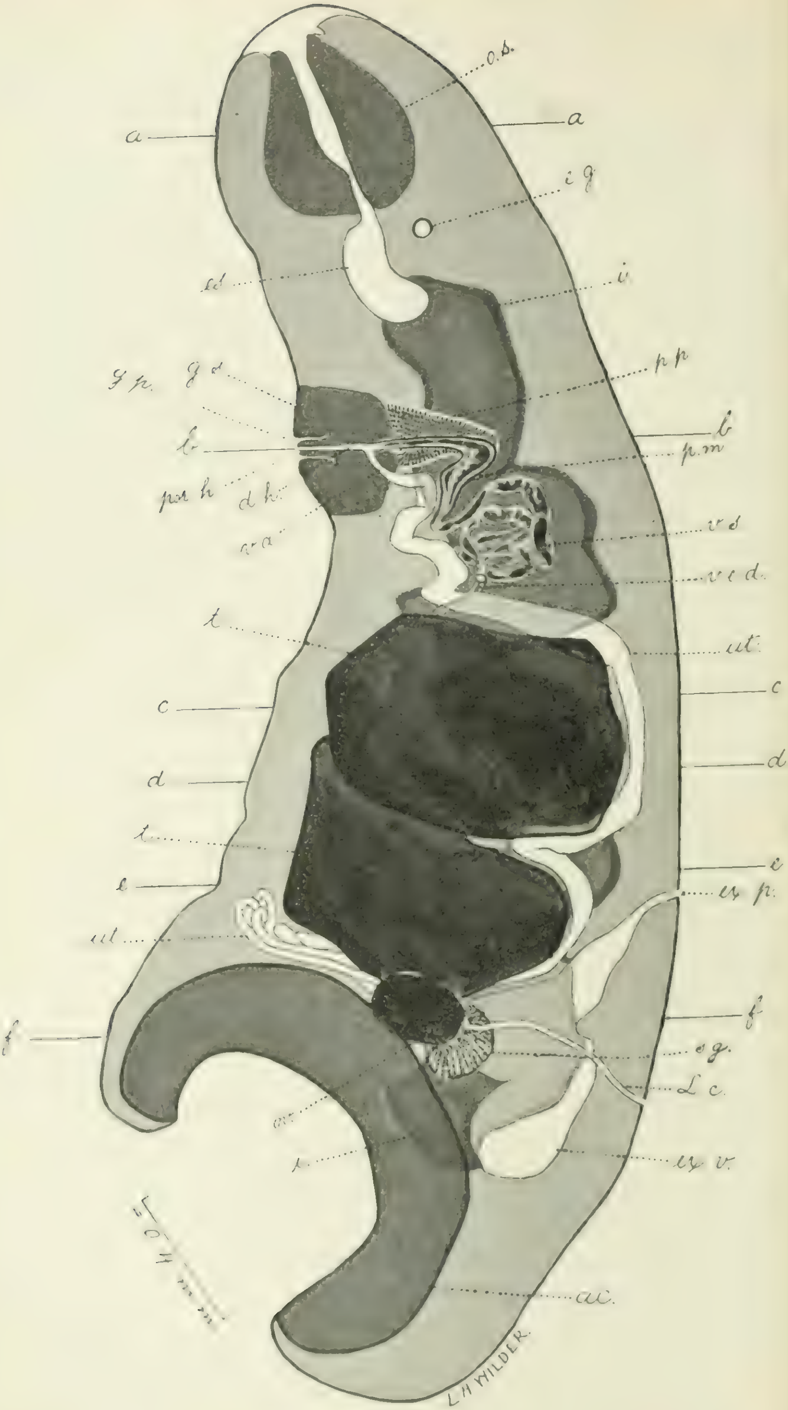


FIG. 44.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L.A. WILDER

FIG. 45.

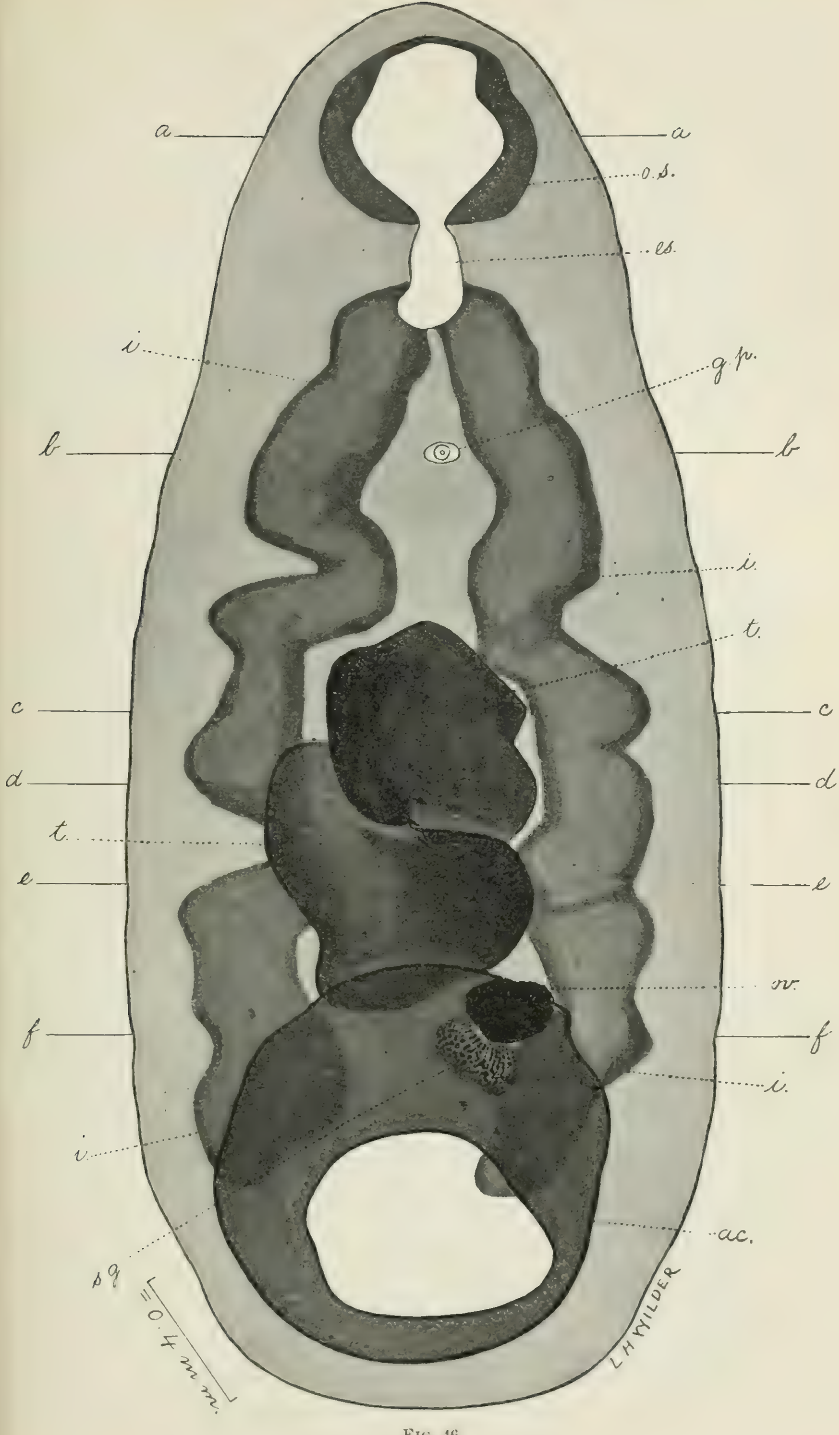

FIG. 46.

The esophagus, from its point of origin at the base of the sucker, passes caudad for a distance equal to about two-thirds the length of the sucker, where it gives off the intestinal ceca from both dorso-lateral aspects. The esophagus describes a slight curve ventrad. Its walls are thick and its lumen is lined with a thick cuticular layer. Dorsally of the first part of the esophagus there is a transverse nervous cord (fig. 45).

The intestinal ceca take their departure from the esophagus at a point slightly less than one-fourth the body length from the oral extremity. At their origin they form a very acute angle one with the other. The ceca at first approach the lateral margins of the body, then pass caudad in a well-marked dorso-ventrally wavy course, to terminate cecally at about the junction of the fifth with the caudal sixth of the body length; the right intestinal cecum terminates at a slightly higher (more cephalic) level than the left. The ceca are of relatively considerable caliber and are lined by a layer of epithelium.

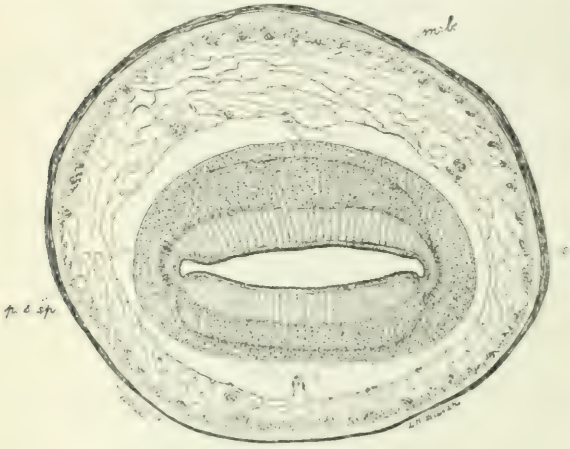

FIG. 47.

GENITAL SYSTEM. — *Male organs.*—The two testes are in the axial region of the body, one caudad of the other, but the caudally placed testis presents a cephalic lobe which overlaps the caudal portion of the right ventro-lateral aspect of the cephalic testis. The two testes occupy a zone immediately cephalad of that of the acetabulum and equal to one-fourth of the body length of the worm. The testes are irregularly globular in form and are indented by fissures marking off numerous lobes on their surface. Each testis gives origin to a vas efferens; that from the cephalic testis springs from its left dorsal aspect (fig. 49), while that of the caudal testis springs from its dorso-cephalic aspect (fig. 51). The vas from the cephalic testis at first passes cephalad close to the left lateral aspect of the testis from which it springs, later it passes cephalo-dorsad and toward the median line, arching over the testis and the uterus to unite with its fellow of the right side (from the caudal testis) to form the vas deferens. The right vas efferens, after its origin from the caudal testis, passes at first to the right and cephalad (dorsally of the cephalic lobe of the caudal testis) to gain the right lateral aspect of the cephalic testis at some slight distance from which it ascends cephalad, eventually arching over the cephalic testis and the uterus to unite, as already described, in forming the vas deferens. The first part of the vas deferens is a much coiled, thin-walled, dilated duct (vesicula seminalis), distended with spermatozoa. This is abruptly succeeded by a narrow, thicker, and more muscular walled uncoiled part (pars musculosa), which ascends cephalad for a short distance, when it in turn is succeeded by a portion which is inclosed in a thick layer of cells. This portion (par prostatica) passes directly ventrad, and after a course about equal in length to the musculosa it penetrates the genital sucker. Here the prostatic cells cease and the continuation of the duct may be regarded as the ductus ejaculatorius. This joins near the base of the genital papilla with the terminal portion of the uterus to form the ductus hermaphroditicus which is 195μ long and pierces the axial region of the genital papilla. The copulatory apparatus presents the genital pore in the median line of the venter at about the junction of the cephalic with the equatorial third of the body and considerably caudad of the esophageal fork (figs. 45, 48). This pore leads into a cylindrical chamber 105μ in depth and about 105μ in diameter, that is almost entirely filled by a cylindrical genital papilla, which, arising from the dorsal wall of the chamber, projects forward (ventrad) into it. The papilla, measured in transverse section, was 75μ in diameter and 105μ in length. Inclosing this genital atrium is a muscular mesh presenting somewhat the form of a hemisphere and in outline suggestive of a sucker. The genital atrium may be regarded as the cavity of the sucker and the genital pore as its aperture. It presents a sharply

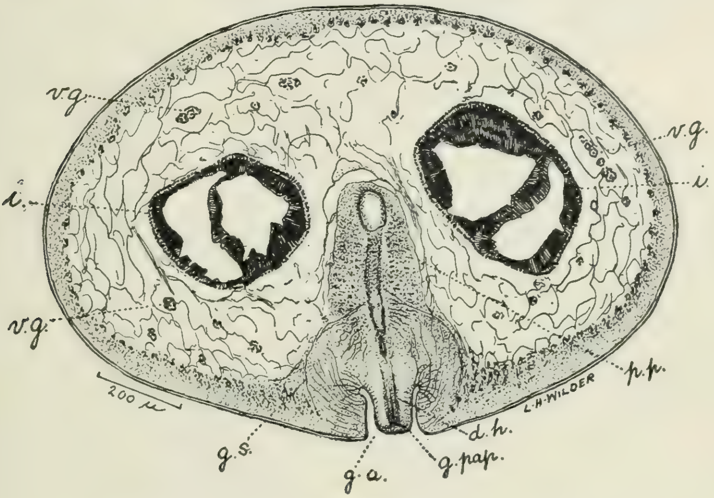

FIG. 48.

defined limiting layer delimiting it from the body parenchyma (fig. 48).

Female organs.—The ovary is in the caudal portion of the body to the left of the median sagittal plane, close to the dome of the acetabulum and immediately caudad of the cephalic margin of the acetabulum. The oviduct takes origin from the dorsal aspect of the ovary and at first passes directly caudad. It then bends and passes to the right toward the shell gland, which it penetrates, but giving off Laurer's canal just before it does so. Immediately after entering the shell gland it unites with the common vitello-duct to form a fusiform ootype, which is directed obliquely from the left and dorsally to the right and ventrally to be continued as the uterus. Laurer's canal passes dorsad close to the left aspect of the excretory vesicle to reach the dorsum at a point to the left of the median line and about 0.56 mm. caudad of the excretory pore.

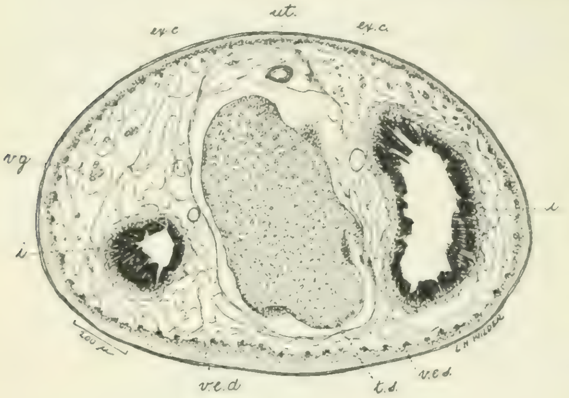

FIG. 49.

The shell gland lies in close apposition to the right side of the ovary; their zones are, however, not identical but overlap, that of the ovary being a little the more cephalad of the two. On account of this relation to the ovary, the shell gland lies just to the left of the median sagittal plane and of the excretory vesicle, which at this level is crowded over to the right. As already stated the shell gland is penetrated by the oviduct, the point of penetration corresponding to the dorsal pole of the gland. It is also penetrated by the common vitello-duct, the point of penetration being close to but a little caudad

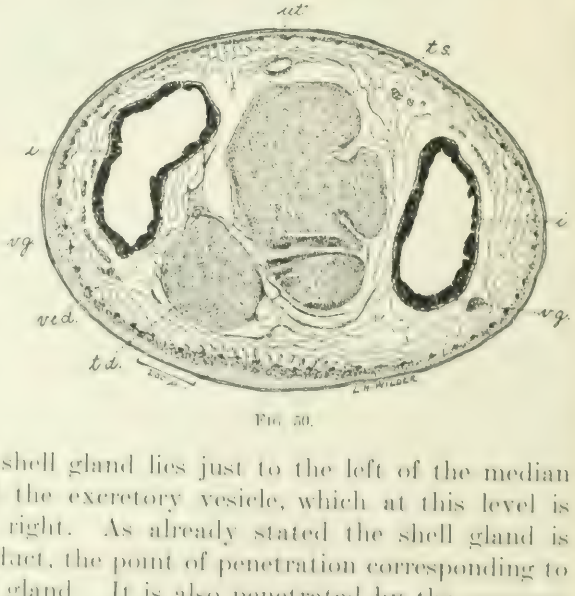

FIG. 50.

of the point of penetration of the oviduct. The uterus emerges from its ventral pole, being, as already described, a continuation of the fusiform ootype. Immediately after emerging, the uterus ascends cephalad, forming some coils close to the right ventro-lateral aspect of the ovary, and ascending further ventro-cephalad it forms some coils directly cephalad of the acetabulum and ventrad of the ventro-caudal aspect of the caudal testicle. It now turns dorsad, skirting the left lateral aspect of the caudal portion of the caudal testis to gain a position dorsally of this testis, where it turns cephalad. The uterus now ascends dorsally of the testes until it reaches the level of the cephalic aspect of the cephalic testis, when it passes ventro-cephalad over this testis and beneath the coiled vesicula and arch of union of the vasa efferentia to gain the ventral aspect of the former. Here it bends cephalad again, eventually penetrating the genital sucker at a point slightly caudad of that of the pars prostatica and, as already described, uniting with the ductus ejaculatorius to form the ductus hermaphroditicus.

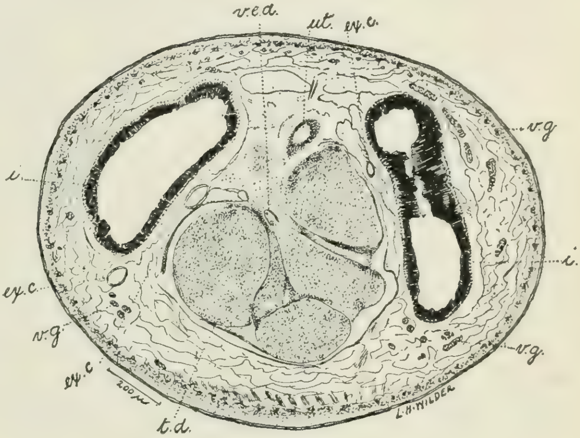

FIG. 51.

The vitellaria, consisting of sparsely scattered but well-developed follicles, are placed in the extracecal fields; longitudinally they are coextensive with the cecal zone, though a few follicles are found slightly caudad of this zone, and not only in the extracecal fields but also in the cecal and intercecal areas.

The transverse vitelloducts pass transversely toward the median line, ventrally of the corresponding testis, and unite close to the ventro-caudal aspect of the shell gland. From their point of union the common vitello-duct takes its departure, skirts closely the caudal aspect of the shell gland and, as already described, penetrates this gland close to its dorsal pole.

Eggs.—Eggs were not observed in the uterus.

EXCRETORY SYSTEM.—The excretory system is well developed. A well-developed excretory vesicle lies in the caudal portion of the body. It is in the form of an inverted elongate pouch, the fundus of the vesicle being close to the dome of the acetabulum in the intercecal field at the level of the cecal ends of the intestines. The body of the vesicle extends cephalad and toward the dorsum, its diameters in transverse section becoming progressively smaller. Eventually it gives place to a short, thick walled duct, which opens in the median line of the dorsum at about the junction of the equatorial with the caudal third of the body length and about 0.16 mm. cephalad of the transverse plane of the cephalic margin of the acetabulum and slightly cephalad of the level of the caudal margin of the caudal testis.

RELATION TO OTHER SPECIES.

Cotylophoron indicum comes close to *C. cotylophorum*, from which it differs chiefly in the structure of the esophagus, which is provided with a bulbus thickening in the latter species but is without it in the former. The two differ also in the details of structure of the copulatory apparatus and in the position of the genital pore. In *C. indicum* the genital sucker is less sharply delimited, projects less, has a much smaller genital atrium, and the genital pore is decidedly postbifurcal; on the other hand, in *C. cotylophorum* the genital sucker is sharply marked, with rim prominently bulging the venter, with a relatively roomy genital atrium and with the genital pore in the bifurcal zone.

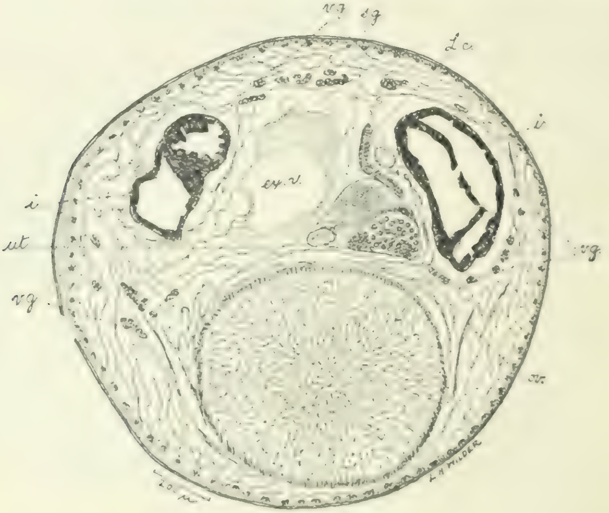

FIG. 52.

ILLUSTRATIONS.

FIG. 43.—Profile view. Enlarged. Original.

FIG. 44.—Ventral view. Enlarged. Original.

FIG. 45.—Profile projection of same, showing oral sucker (*o. s.*), esophagus (*es.*), esophageal ganglion (*e. g.*), right intestine (*i.*), genital sucker (*g. s.*), testes (*t.*), right vas efferens (*v. e. d.*), vesicula seminalis (*v. s.*), pars musculosa (*p. m.*), pars prostatica (*p. p.*), ductus hermaphroditicus (*d. h.*), porus hermaphroditicus (*por. h.*), genital pore (*g. p.*), metraterm (*va.*), uterus (*ut.*), shell gland (*s. g.*), ovary (*ov.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v*), excretory pore (*ex. p.*), and acetabulum (*ac.*). *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 46.—Ventral projection of same. Lettering as in fig. 45. Slightly diagrammatic. Enlarged. Original.

Fig. 47.—Transverse sections at *a–a*, figs. 45 and 46. Shows oral sucker (*o. s.*) with the papillæ lining the lumen, perisuctorial space (*p. s. sp.*), and mesenterial band (*m. b.*). Enlarged. Original.

Fig. 48.—Transverse section at *b–b*, figs. 45 and 46. Shows genital atrium (*g. a.*), genital papilla (*g. pap.*), genital sucker (*g. s.*), ductus hermaphroditicus (*d. h.*), pars prostatica (*p. p.*), intestinal ceca (*i*), vitellaria (*v. g.*). Enlarged. Original.

Fig. 49.—Transverse section at *c–c*, figs. 45 and 46. Shows form of body at equator, cephalic testis (*t. s.*), origin of left vas efferens (*v. e. s.*), position of the right vas efferens (*v. e d.*), uterus (*ut.*), intestinal ceca (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 50.—Transverse section at *d–d*, figs. 45 and 46. Shows caudal portion of cephalic testis (*t. s.*), and cephalic lobe of caudal testis (*t. d.*), right vas efferens (*v. e. d.*), uterus (*ut.*), intestinal ceca (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 51.—Transverse section at *e–e*, figs. 45 and 46. Shows caudal testis (*t. d.*), origin of right vas efferens (*v. e. d.*), uterus (*ut.*), intestinal ceca (*i.*), vitellaria (*v. g.*), and excretory canals (*ex. c.*). Enlarged. Original.

Fig. 52.—Transverse section at *e–e*, figs. 3 and 4. Shows ovary (*ov.*), shell gland (*s. g.*), first part of uterus (*ut.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), intestinal ceca (*i.*), vitellaria (*v. g.*), and acetabulum (*ac.*). Enlarged. Original.

Genus **PARAMPHISTOMUM**, Fischœder, 1901.

Generic diagnosis.—*Paramphistominæ* (p. 62): Body tends to a conical form, with convex dorsum and cancave venter, rather attenuate cephalad, rather blunt caudad; ventral pouch absent. Acetabulum terminal, tilts ventrad, small to very large, sunken, margin not raised, aperture small to large. *Genital sucker absent*, pore ventro-median, pretesticular. Excretory vesicle at least partly in acetabular zone. *Oral sucker without evagination;* esophagus with or without muscular thickening; ceca long, nearly straight to wavy, end postequatorial, posttesticular, usually in acetabular zone.

Male organs: Testes 2, usually interceeal, small to large, finely lobulate to coarsely lobate, exceptionally nearly smooth, fields coincide to separate, zones coincide to separate; *cirrus pouch absent.*

Female organs: Ovary and shell gland usually posttesticular, never pretesticular; Laurer's canal may cross vesicle; uterus runs dorsally of testes, under arch of vasa efferentia, then ventrally of vas deferens.

Eggs: With (or without ? ?) operculum.

Type species.—*P. cervi* (Schrank, 1790).

The species of this genus may be arranged in several groups, the individual members of which appear more closely related to each other than to individual members of other groups. Still it is somewhat difficult at present to separate the forms with entire satisfaction. That several of these groups will eventually be recognized as of generic rank seems certain, but in the interest of conservatism, we propose to recognize them temporarily only as subgenera, as follows:

Subgenus *Paramphistomum*, with *cervi* (type), *epiclitum*, *liorchis*, and *bathycotyle*.
Subgenus *Orthocœlium*, with *orthocœlium* (type), and *dicranocœlium*.
Subgenus *Bothriophoron*, with *bothriophoron* (type), and *papilligerum*.
Subgenus *Cauliorchis*, with *cauliorchis* (type), *crassum*, probably *papillosum*, and doubtfully *indicum* and *calicophorum*.
Subgenus uncertain: All the other known species.

The following key will aid in separating the species in question:

Key to species of Paramphistomum

A^2. Laurer's canal crosses excretory canal or vesicle..............................p. 75.

A^1. Laurer's canal does not cross excretory canal or vesicle, its pore is always cephalad of excretory pore; ovary prevesicular; testes touch median line:

  B^1. Ceca nearly or quite straight, end in pre- or acetabular zone; esophagus with slight muscular thickening; acetabulum small; excretory vesicle club-shaped; ventral chamber of genital atrium absent; testes lobate, distinctly and considerably preacetabular, fields coincide; vitelline follicles coarse, grouped; type *orthocœlium*..............................Subgenus *Orthocœlium*, p. 77.

    C^1. Excretory vesicle and canal directed dorso-cephalad, excretory pore slightly prevesicular; genital pore bifurcal; vitelline follicles usually in single row each side; testes about as large as acetabulum; oral sucker with sphincter; esophagus twice as long as oral sucker; ceca deep, end pre- to acetabular; body 5 to 11 mm. long; type host *Bos kerabau*, from Ceylon.
P. orthocœlium, p. 77.

    C^2. Excretory vesicle and canal directed dorso-caudad, excretory pore postvesicular; genital pore postbifurcal; vitelline follicles usually in two rows each side; testes larger than acetabulum; oral sucker without sphincter; esophagus about one and one-half times as long as oral sucker; ceca rather narrow and in section nearly circular, end preacetabular; body 5 to 8 mm. long; type host *Bos indicus*..........................*P. dicranocœlium*, p. 77.

  B^2. Ceca sinuous to wavy, end in acetabular zone; [subgeneric position as yet uncertain, but not typical subgenus *Paramphistomum*]:

    C^3. Esophageal muscular thickening absent; esophagus about as long as sucker; ceca wavy; excretory pore in vesicular zone; acetabulum terminal small to moderate; genital pore postbifurcal; ventral chamber of genital atrium small; testes (? about equal to and) removed from acetabulum, fields coincide, zones slightly separate; musculosa long, coiled; ovary slightly ventro-cephalic; vitellaria in esophageal, cecal, and cephalic part of postcecal zone; body 5 to 11 mm. long; type host *Bos kerabau*, Ceylon.
P. streptocœlium.

    C^4. Esophageal muscular thickening present; testes lobate; acetabulum moderate:

      D^1. Excretory pore prevesicular; ventral chamber of genital atrium present, genital atrium (dorsal chamber) papillated; ceca wavy; testicular zone overlaps acetabulum; body 3 to 3.9 mm. long, 1.8 to 2.3 mm. broad; type host *Bos indicus*, Siam..................*P. parvipapillatum*, p. 143.

*D*². Excretory pore in vesicular zone; ventral chamber of genital atrium small, complicated; musculosa quite large, coiled; ceca wavy; testicular zone slightly preacetabular, testes slightly larger than acetabulum, testicular fields nearly coincide; ovary acetabular; vesicle not club-shaped; body between 4.5 and 7 mm. long, 2.46 mm. broad, 2.26 mm. thick; type host *Cervus eldi*................................*P. shipleyi*, p. 150.

*D*³. Excretory pore postvesicular; ventral chamber of genital atrium absent; ceca sinuous; testicular zones abut to separate, slightly preacetabular; testicular fields coincide; vitelline follicles coarse, grouped, extend esophageal, cecal, postcecal; vesicle not club-shaped; body 2 to 6 mm. long, 0.7 to 2.5 mm. broad; type host *Bos bubalus*, Cochin China, and *B. taurus*, Annam................................*P. scoliocœlium* Fischœder, 1904.

*A*². Laurer's canal crosses excretory canal or vesicle; Laurer's pore (except in *cauliorchis* and possibly *papilligerum*) caudad of excretory pore; ovary never entirely cephalic of vesicle, but always ventral; testes not distinctly larger, but usually smaller than or about equal to acetabulum; esophagus without muscular thickening; ceca sinuous to wavy, not straight:

*B*³. Ventral chamber of genital atrium very large; testes lobate, about equal in size to acetabulum, testicular fields median, nearly coincide, zones slightly separate, nearly abut, preacetabular; acetabulum moderate; ceca long, end in acetabular zone, moderately broad; type *bothriophoron*.
Subgenus *Bothriophoron*, p. 77.

*C*⁵. Ventral chamber not papillate; pore of Laurer's canal latero-caudad of excretory pore, which is prevesicular; testes a little removed from acetabulum; genital sphincter present; body 6 to 9 mm. long; type host *Bos indicus*, Madagascar......................................*P. bothriophoron*, p. 77.

*C*⁶. Ventral chamber papillate; pore of Laurer's canal slightly caudad of excretory pore; testes slightly preacetabular; body 8.5 mm. long, 4.3 mm. broad; type host *Cervus eldi*................................*P. papilligerum*, p. 78.

*B*⁴. Ventral chamber of genital atrium absent or small:

*C*⁷. Acetabulum large to very large:

*D*⁴. Excretory pore prevesicular:

*E*¹. Testicular fields separate, not median, zones overlap, testes lobate, much smaller than acetabulum, acetabular to somewhat preacetabular but not far removed; musculosa long and thick; Laurer's pore postero-lateral of excretory pore; ventral chamber (?); genital pore postbifurcal, excretory vesicle not narrow, but swollen; acetabulum large; body 5 to 8 mm. long; type hosts *Bos taurus*, Togo, *Bos zebu*, German East Africa.................*P. calicophorum*, Fischœder, 1901.

*E*². Testicular fields median, coincide or overlap, zones lobate, testes much smaller than and near the acetabulum; ventral chamber absent; excretory vesicle long and narrow; acetabulum very large:

*F*¹. Genital pore in suctorial zone; musculosa straight, narrow, prostatica shorter than musculosa; acetabulum less than half as long as body; testes large; ceca wavy, broad; excretory vesicle long; body 9.75 mm. long, 4.5 mm. broad; type host *Buffelus indicus*.
P. fraternum, p. 131.

*F*². Genital pore in esophageal zone; musculosa coiled; testes large; acetabulum more than half as long as body; ceca wavy, moderately broad, end in acetabular zone; body 6 to 9 mm. long, 4 mm. broad; type host *Bos indicus*, Siam.....................*P. siamense*, p. 161.

*F*³. Genital pore in postbifurcal zone; musculosa (?); prostatica longer than musculosa; acetabulum less than half as long as body; ceca sinuous, moderately broad, end in acetabular zone; body 8 to 13 mm. long; type host *Bos indicus* at Berlin, Germany.
P. explanatum Creplin, 1847.

D^5. Excretory pore in vesicular zone; testes small, much smaller than acetabulum, cauliflower shaped, near acetabulum; vesicle bag-like; ceca wavy:

E^3. Testicular fields separate, not median, testes pre- and acetabular; musculosa enormous; vasa efferentia convex cephalad; ovary ventro-cephalic of vesicle; pore of Laurer's canal slightly latero-cephalic of excretory pore; genital pore postbifurcal; ceca moderately broad; body 6 to 7.5 mm. long, 3.6 to 4.25 mm. broad; type host *Bos indicus*, India...*P. cauliorchis*, p. 86.

E^4. Testicular fields overlap, median; testes diagonal, pre- and acetabular, musculosa large; ovary ventral of vesicle; pore of Laurer's canal caudad of excretory pore; genital pore in suctorial zone; ceca broad; body 6 to 6.5 mm. long, 4 to 4.76 mm. broad; type host *Bos indicus*, India...*P. crassum*, p. 101.

E^5. Testicular fields coincide, median, testes preacetabular; musculosa large; ovary ventro-cephalic of vesicle; pore of Laurer's canal caudad of excretory pore; ventral chamber small, papillate; genital pore in esophageal zone, ceca moderately broad; body 5 to 6 mm. long, 2.6 mm. broad; type host *Bos indicus*, India.......*P. papillosum*, p. 112.

C^5. Acetabulum small to moderate; ventral chamber absent; testicular fields median, coincide or overlap; ovary ventral of vesicle:

D^6. Testes distinctly removed from acetabulum; genital pore postbifurcal; vesicula club-shaped; excretory pore prevesicular:

E^6. Acetabulum small, about one-eighth as long as body; genital sphincter absent; pars prostatica large, oval; musculosa short, not coiled; body 11 to 15 mm. long; type host *Bos kerabau*, also in *Portax tragocamelus*.................................*P. gracile* Fischœder, 1901.

E^7. Acetabulum moderate; genital sphincter present; pars prostatica thin; musculosa long, coiled; body 8 to 11 mm. long; type host *Antilope dorcas*..............................*P. microbothrium* Fischœder, 1901.

D^7. Testes near acetabulum:

E^8. Testes much smaller than acetabulum, cauliflower-like; excretory vesicle bag shaped, distended; excretory pore in pre- and vesicular zones; body 5.25 to 9.5 mm. long, 2 to 3 mm. broad; type host *Bos indicus*, India...*P. indicum*, p. 121.

E^9. Testes about equal to (or slightly smaller or larger than) acetabulum; excretory pore distinctly prevesicular; type *cervi*.

Subgenus *Paramphistomum*, p. 77.

F^1. Testes nearly smooth; genital pore prebifurcal; pars musculosa very short; body 3 to 8 mm. long; type hosts *Cervus simplicicornis*, *C. campestris*, *C. mexicanus*, *C. rufus*, *C. dichotomus*, *C. nambu*, Brazil..................................*P. liorchis* Fischœder, 1901.

F^2. Testes lobate:

G^1. Testes distinctly smaller than acetabulum; acetabulum one-third as long as body; body 11 to 15 mm. long; type host *Bos kerabau*, Ceylon..........................*P. bathycotyle* Fischœder, 1901.

G^2. Testes not distinctly smaller than acetabulum:

H^1. Genital pore bifurcal or only slightly postbifurcal; body 5 to 12 mm. long; type host *Cervus elaphas*, Europe..*P. cervi* (Schrank, 1790).

H^2. Genital pore markedly postbifurcal; body 5 to 9 mm. long, 2 to 3 mm. broad; type hosts *Bos bubalus*, Cochin-China, and *Bos indicus*, at Berlin, Germany...................*P. epiclitum* Fischœder, 1904.

Subgenus PARAMPHISTOMUM.

Subgeneric diagnosis.—*Paramphistomum* (p. 73): Ceca sinuous to wavy, not straight; long, end in or close to acetabular zone; esophagus without muscular thickening. Acetabulum moderate, terminal. Excretory vesicle elongate, more or less club shaped, its pore prevesicular. Ventral chamber of genital atrium absent.

Male organs: Testes lobate to smooth, near and about equal to acetabulum, at least neither very much larger nor very much smaller (except possibly *bathycotyle*), testicular zones slightly separate or abut or slightly overlap, fields median, nearly or quite coincide.

Female organs: Ovary ventral of vesicle; Laurer's canal crosses excretory canal or vesicle, its pore caudad of excretory pore; vitellaria in esophageal, cecal, and postcecal zones.

Type species.—*P. cervi* (Schrank, 1790).

This subgenus contains at least 4 species (*cervi*, *epiclitum*, *liorchis*, and *bathycotyle*), all of which have been studied by Fischœder.

ORTHOCŒLIUM, new subgenus.

Subgeneric diagnosis.—*Paramphistomum* (p. 73): Ceca nearly or quite straight, end preacetabular or in proximal portion of acetabular zone; esophagus with slight muscular thickening. Acetabulum small, terminal, tilts ventrad. Excretory vesicle club shaped. Ventral chamber of genital atrium absent.

Male organs: Testes about equal to or larger than acetabulum, lobate, zones abut and are considerably preacetabular, fields coincide, in median line.

Female organs: Ovary prevesicular; Laurer's canal does not cross excretory vesicle or duct, its pore cephalad of excretory pore; vitelline follicles coarse, grouped, extend from esophageal into postcecal and even into acetabular zone.

Type species.—*Paramphistomum orthocœlium* Fischœder, 1901.

This subgenus, which at present contains two species (*orthocœlium* and *dicranocœlium*, see key p. 74), will probably eventually be recognized as a distinct genus. Both of these species have been studied by Fischœder.

BOTHRIOPHORON, new subgenus.

Subgeneric diagnosis.—*Paramphistomum* (p. 73): Ceca sinuous to wavy, not straight; long, end in acetabular zone, and moderately broad; esophagus without muscular thickening. Acetabulum moderate, terminal. Excretory vesicle distended, not club shaped. *Ventral chamber of genital atrium very large.*

Male organs: Testes lobate, about equal in size to and not far away from acetabulum, testicular zones slightly separate, nearly abut, fields median, nearly coincide.

Female organs: Ovary ventral of vesicle; Laurer's canal crosses excretory canal or vesicle, its pore (in the type species at least) caudad of the excretory pore; vitellaria well developed, extend from bifurcation to slightly caudad of ceca.

Type species.—*Paramphistomum bothriophoron* (Braun, 1891).

Two species (namely, *bothriophoron* and *papilligerum*) may be classified here. They are easily separated by the papillate or nonpapillate condition of the ventral chamber of the genital atrium. *P. bothriophoron* has been recently studied by Fischœder.

PARAMPHISTOMUM PAPILLIGERUM, new species.

[Figs. 53 to 56.]

SPECIFIC DIAGNOSIS.—*Paramphistomum* (p. 73): Body 8.5 mm. long by 4.3 mm. broad; color (?); greatest breadth near caudal end; tapers gradually to bluntly pointed oral pole, very rapidly to very bluntly rounded caudal pole. Surface without papillæ. Genital pore at junction of cephalic with equatorial thirds of body, with elliptical bulging 1.08 mm. in transverse, 0.8 mm. in longitudinal diameter, with 2 transverse labia; genital pore about 0.61 mm. by 0.37 mm.; surface of bulging sparsely papillate; at this point a crateriform, papillate atrium. Acetabulum terminal, sunken in parenchyma, its margin not projecting, 1.88 mm. in transverse diameter, opening caudad (terminal) 0.46 mm. in transverse diameter. Mouth in depression at cephalic pole; oral sucker rather oval in frontal section, its lumen papillate; lies in a well developed perisuctorial cavity; esophagus bent, convexity ventrad, its wall rather uniform in thickness; ceca long, wavy dorso-ventrally, extend caudad about to equator of acetabulum. Excretory pore about dorso-median, slightly cephalad of pore of Laurer's canal; excretory canal runs to dorsal (or dorso-caudal?) aspect of well-developed vesicle lying dorsally and dorso-cephalad (?) of acetabulum.

Male organs: Testes large, lobate, in median line, interceecal, fields overlap, zones slightly overlap; union of vasa efferentia slightly cephalad of cephalic testis; vas deferens dilated, coiled; pars intermedia connects this with coiled musculosa; pars prostatica relatively short, passes directly ventrad; ductus ejaculatorius opens just above metraterm into transverse (in frontal plane) slit-like chamber, whence a duct passes ventrad apparently piercing a papilla; the latter projects into a chamber which is connected by a very short duct with the papillated genital atrium.

Female organs: Ovary and shell gland between caudal testis and acetabulum, ovary slightly to left of median line, shell gland about median; vitellaria with well-developed, sparsely scattered follicles, chiefly in extraceecal area, extending from esophageal to slightly caudad of cecal zone; uterus extends from shell gland slightly caudo-sinistrad, then ventrally of ovary and shell gland dextrad, bends cephalad dorsally of testes, ventrad under arch of vasa efferentia, cephalad ventrally of vas deferens to open caudad of male opening into the slit-like chamber.

Eggs: Oval, 135μ by 67μ, operculated (?).

TYPE.—U.S.P.H. & M.H.S. 10706 (returned to Doctor Shipley).

HABITAT.—In (stomach (?) of) *Cervus eldi*, locality not known.

SOURCE OF MATERIAL.—The material, consisting of a series of frontal sections, was kindly loaned us by Dr. A. E. Shipley.

EXTERNAL CHARACTERS.

SIZE.—Measurements taken from the sections give about 8.5 mm. for the maximum vertical diameter and about 4.3 mm. for the maximum transverse diameter.

FORM.—The greatest width of the worm is near the caudal extremity, from which region it tapers in both directions. The caudal extremity is broad, rounded from side to side, and presents the aperture of the acetabulum; the cephalic extremity is bluntly pointed and presents at its vertex a well-marked depression, at the bottom of which is the oral aperture.

SURFACE.—The general surface of the worm appears unprovided with such structures as spines or hooks, and except for the genital bulging is without papillæ.

Genital pore.—In about the median sagittal line of the venter and about at the junction of the cephalic with the middle third of the body length there is a bulging, elliptical in outline and measuring at the base about 1.08 mm. in transverse and about 0.80 mm. in vertical diameter. At the vertex of this bulging there are two transverse lip-like structures, continuous laterally, which bound, as it were, a transversely elliptical aperture. This aperture may be regarded as the genital pore, and measures 0.61 mm. in transverse and about 0.37 mm. in median sagittal diameter. The surface of the bulging is beset by minute sparsely scattered papillæ.

The genital pore gives entrance to a crateriform depression or atrium, the surface of which is closely beset by nipple-like papillæ (fig. 53), which are considerably larger than those of the surface of the genital bulging.

Acetabulum.—The acetabulum is in the caudal extremity of the worm and measures 1.88 mm. in greatest transverse diameter. Its aperture is terminal and measures 0.46 mm. in maximum transverse diameter. The rim of the acetabulum does not project beyond the caudal body surface, but appears rather retracted and its aperture slightly encroached upon by folds of the encircling portions of the body.

INTERNAL ANATOMY.

Digestive tract.—The mouth is at the bottom of the depression at the cephalic pole of the worm, and leads directly into the lumen of a large muscular oral sucker.

In frontal section the sucker has an oval form with a broad rounded base, which gives origin to the esophagus. The oral pole of the sucker projects in a ring-like manner beyond the adjacent surface. A roomy perisuctorial space is present. The lumen of the sucker is closely beset by well-marked conical papillæ. The esophagus at first passes directly caudad in about the median sagittal plane, then bends ventrad and describes a short curve, or possibly forms only a moderate bend (the interpretation is difficult) with its convexity ventrad. It then passes caudo-dorsad and forks into the lateral intestinal ceca at or somewhat cephalad of the level of the genital pore. This relation could not be made out with precision from the sections. The thickness of the esophageal wall is substantially uniform throughout. Its lumen is lined with a cuticle-like layer. In about the equator of the esophagus and dorsally of it there is a transverse ganglionic cord. The intestinal ceca pass at first laterad and slightly cephalad at or slightly less than a right angle with the esophagus, then turn caudad pursuing a dorso-ventrally wavy course approximately parallel to the lateral body wall, terminating by blind extremities about 5 mm. caudad of the esophageal arch close to the corresponding dorso-lateral aspect of the acetabulum.

GENITAL SYSTEM.—The sexual organs, with the exception of the vitellogene glands, are disposed in the intercecal area.

Male organs.—There are two lobate testes, one caudad of the other, their opposing aspects being in close apposition. The cephalically placed of the two is a little nearer the left and the caudally placed a little nearer the right lateral body margin. In some of the sections the testes appear somewhat wedge-shaped, the left edge of the cephalic testis and the right of the caudal testis being (vertically) the longer of the vertical edges of the 2 testes. As the testes are in fairly close apposition, the line of separation between them runs somewhat obliquely from the right to the left and caudad, and consequently their zones overlap to a slight extent (fig. 54). A vas efferens springs from each testis; that from the caudal gland passes cephalad with a slight tilt dorsad between the cephalic testis and the right gut, and is of considerable transverse diameter; that from the cephalic testis passes at first dorsad, then to the left and cephalad. The latter is of a smaller caliber and soon reaches the upper part of the space between the dorsolateral aspect of the gland from which it springs and the left gut, and then dilating rather abruptly it curves cephalo-mediad to unite with its fellow of the right side, which likewise curves cephalo-mediad to meet it, and the two thus form the vas deferens. The vasa efferentia in uniting form a transverse arch at a level apparently slightly cephalad of that of the superior margin of the cephalic testis; beneath this arch the uterus is seen to pass as it arches cephalo-ventrad over the cephalic testis. The vas deferens presents at first a much dilated, thin-walled, coiled vesicula measuring about 0.27 mm. in diameter; this is succeeded by a long, thick-walled, coiled pars musculosa of about 180μ in diameter and with a thickness of wall of about 60μ. Between the vesicula and the musculosa there is intercalated a relatively thick-walled segment measuring about 75μ in diameter. The musculosa is succeeded by a relatively short prostatica; this part of the vas deferens is inclosed in a thick layer (75μ) of prostatic cells and passes directly ventrad. The prostatic

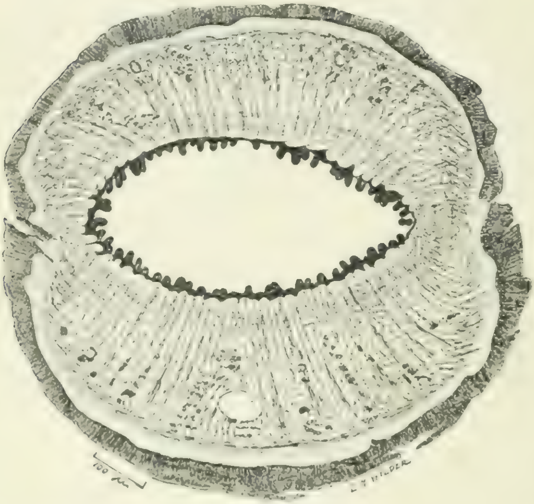

FIG. 53.

portion is succeeded by a ductus ejaculatorius which, with the metraterm, is inclosed in a thin layer of an ill-defined muscular mesh and opens with, but just above, the metraterm into a frontally transverse slit-like chamber. From this chamber a duct passes ventrad, apparently piercing a papilla, the form of which can not satisfactorily be made out. This papilla projects ventrad into a chamber (from the dorsal wall of the latter as it were) so that the duct piercing it opens into this chamber. This chamber in its turn communicates with the crateriform, papillated ventral genital chamber (previously described) by

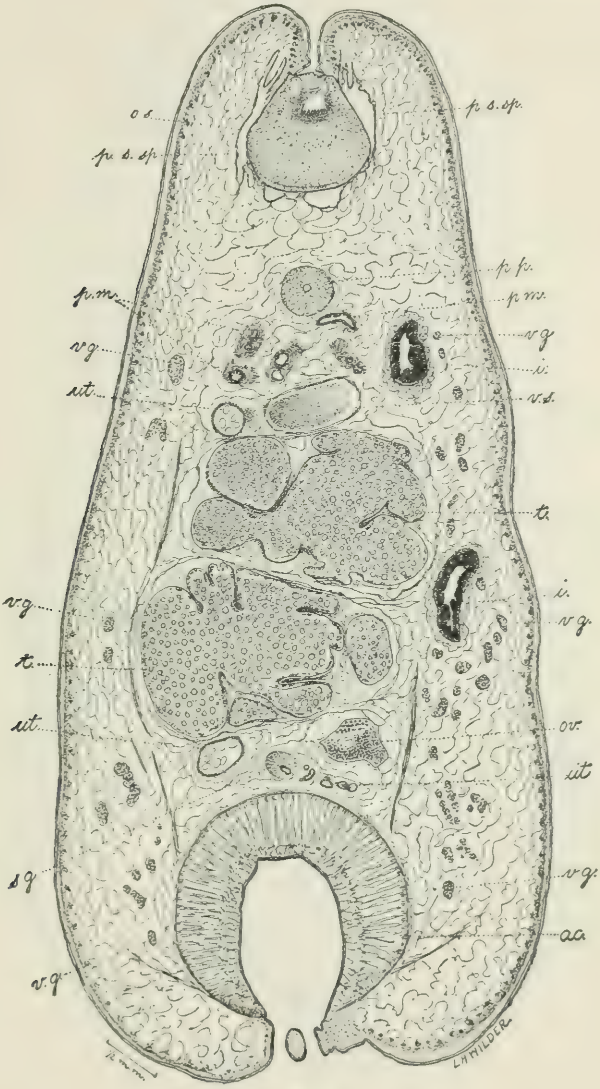

FIG. 54.

a very short and quite narrow duct. This series of atria and connecting ducts of the hermaphroditic copulatory apparatus seems to resemble that of *Paramphist. shipleyi*. The marked and striking difference between the two, so far as one can judge from the sections avail-

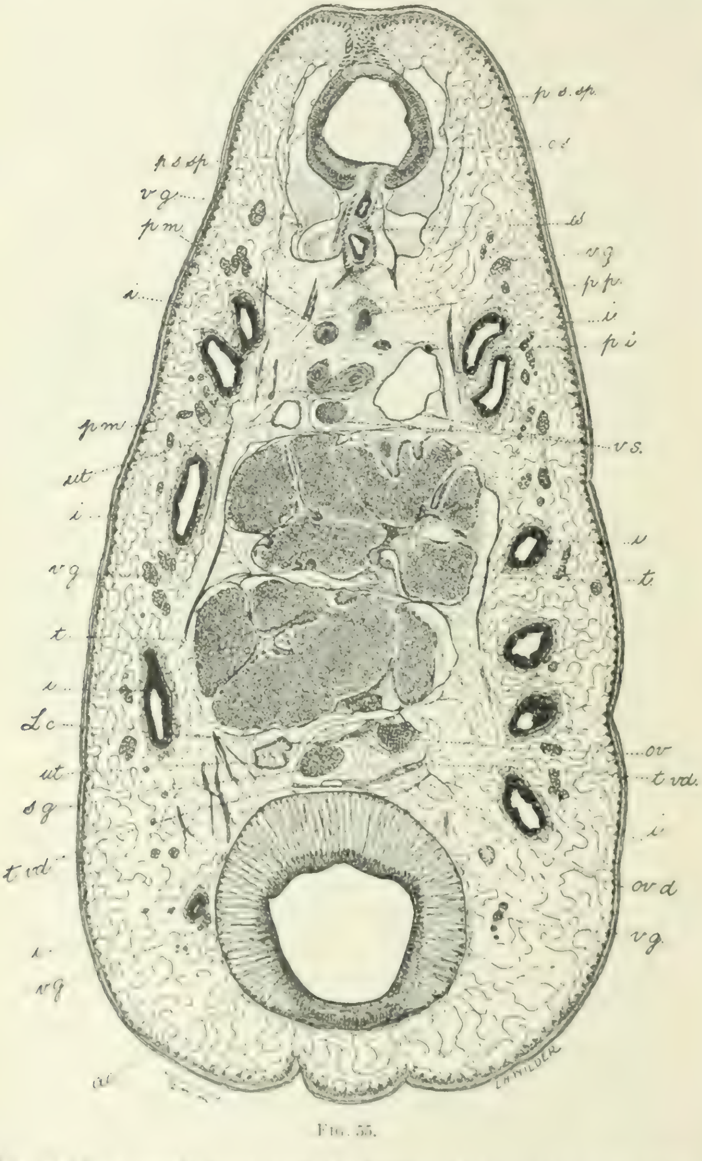

FIG. 55.

able for study, consists in the difference (1) in the size and form of the genital pore (that of *P. papilligerum* is large, elliptical, and measures 610μ in transverse diameter, while that of *P. shipleyi* is small, approximately circular, and measures about 75μ in transverse diameter); and (2) in the size and form of the ventral chamber of the genital atrium), which in *P. papilligerum* is large crateriform,

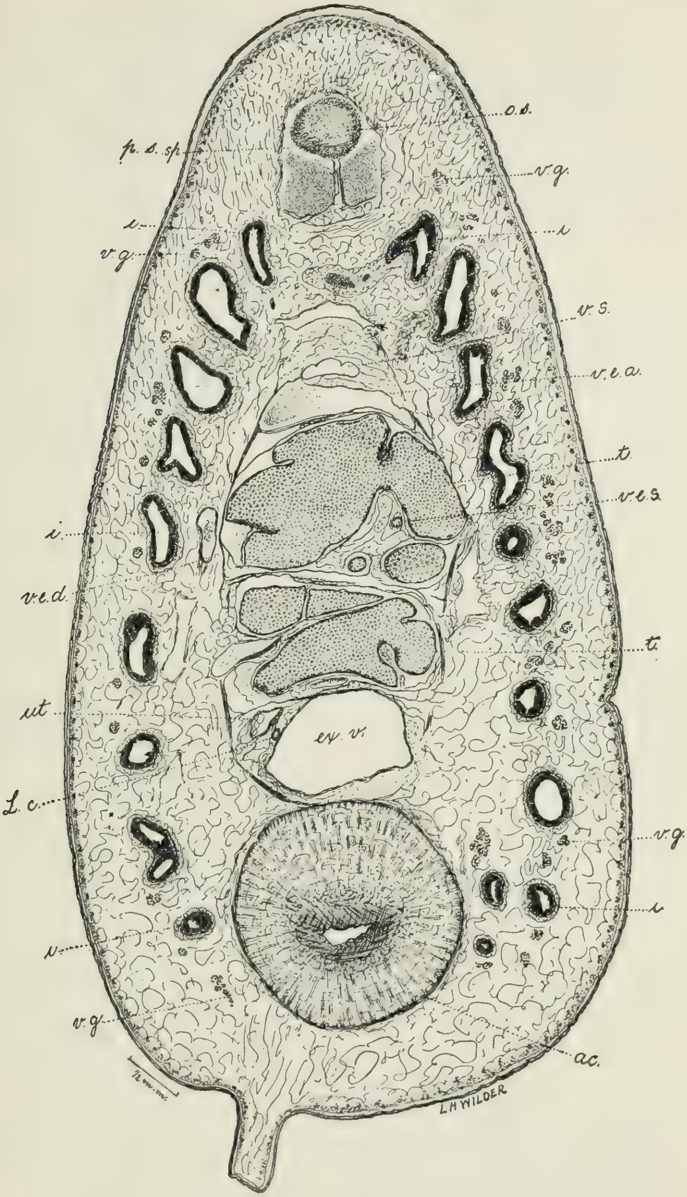

FIG. 56.

with its surface beset by well developed nipple-like papillæ, while in *P. shipleyi* the ventral chamber is small, slit-like, and without papillæ on its surface.

Female organs.— The ovary and shell gland are in the caudal portion of the worm, between the caudal testis and the acetabulum, and nearer the venter than the dorsum. The ovary is the larger of the two female glands and is also the nearer to the venter and is placed a little to the left of the median sagittal plane. The oviduct springs from the dorsal pole of the ovary; it passes to the right, curving gently caudad as it proceeds toward the shell gland, close to the cephalic aspect of which it forks. One limb of the fork, which may be regarded as the continuation of the oviduct, turns sharply caudad to penetrate the shell gland; the other limb bends dorso-caudad and, as Laurer's canal, at first skirts the right side of the dome of the excretory vesicle, but as it tends more and more caudad it crosses the right side of the vesicle and eventually reaches the dorsum a little to the right of the median sagittal plane and about, or perhaps a little less than 0.52 mm. caudad of the excretory pore. The shell gland is close to the right caudo-lateral aspect of the ovary, close to the ventro-cephalic aspect of the excretory vesicle, and in about the median sagittal plane of the worm.

As already described, it is penetrated on its cephalic aspect by the oviduct which unites in the substance of the gland with the common vitello-duct. The latter duct penetrates the gland at its caudal aspect. The union of these ducts results in the formation of the ootype, which is directed ventrad in the substance of the gland. The ootype is continued as the uterus, which emerges from the ventral pole of the gland. After emerging, the uterus dips caudad and to the left, then doubling back it forms coils ventrally of the ovary and shell gland, then winds its way dorsad to the right of the shell gland beneath the caudal testis, close to and on the right of Laurer's canal. On reaching the space between the caudal testis and the dorsum it begins to wind its way cephalad. Eventually it reaches the level of the superior margin of the cephalic testis, where it bends ventrad, passing, as already mentioned, beneath the arch of union of the vasa efferentia to gain the ventro-lateral aspect of the coiled vas deferens. Here it bends cephalad, ascending in close relation to the vas deferens, ultimately arching ventrad close to the caudal aspect of the pars prostatica and ductus ejaculatorius to open, as has been described, just beneath the latter into a small slit-like chamber. The uterus is distended more or less in various portions of its course by eggs and by masses of spermatozoa. The eggs are oval in form; some of them measured 135μ in length by 67μ in width.

The vitellaria consist of sparsely scattered well developed follicles in the extracecal areas. Vertically they extend from about the level of the base of the oral sucker to or perhaps slightly caudad of the level of the cecal ends of the gut. Their zone, therefore, is about coextensive with the combined zones of the esophagus and intestinal ceca. A transverse vitello-duct from each gland passes mediad ventrally of the corresponding intestine. These ducts unite close to the ventro-caudal aspect of the shell gland. The common vitelloduct thus formed passes dorsad close to the shell gland, the caudal aspect of which it eventually penetrates.

EXCRETORY SYSTEM.—This seems well developed. A large vesicle lying dorsad of the acetabulum is present. An excretory duct leaves the dorsal, probably caudo-dorsal, aspect of the vesicle and passes to about the median vertical line of the dorsum to open at a point in a transverse plane slightly cephalad of that of the pore of Laurer's canal.

RELATION TO OTHER SPECIES.

This species appears most nearly related to *Param. bothriophoron*, from which it differs mainly in the structure of the copulatory apparatus. In *P. papilligerum* the genital chamber is papillated and the genital pore is without a sphincter; in *P. bothriophoron* the chamber is not papillated and the genital pore is encircled by a sphincter. Besides this, large papillæ line the suctorial lumen of *P. papilligerum;* they are absent in *P. bothriophoron.*

ILLUSTRATIONS.

FIG. 53.—Optical section through genital bulging near its vertex. Shows papillæ in genital atrium. Enlarged. Original.

FIG. 54.—Optical section. Shows depression at oral pole leading to the mouth; oral sucker (*o. s.*) with papillæ, perisuctorial space (*p. s. sp.*), pars prostatica (*p. p.*), pars musculosa (*p. m.*), vesicula seminalis (*v. s.*), uterus (*ut.*), lobate testes (*t.*), ovary (*ov.*), shell gland (*s. g.*), intestine (*i*) of left side, vitellaria (*v. g.*), and acetabulum (*ac.*). Enlarged. Original.

FIG. 55.—Optical section. Shows oral sucker and papillæ (*o. s.*), perisuctorial space (*p. s. sp.*), esophagus (*es.*), point of change from musculosa to prostatica (*p. p.*), pars musculosa (*p. m.*), pars intermedia (*p. i.*), vesicula seminalis (*v. s.*), uterus (*ut.*), testes (*t.*), ovary (*ov.*), oviduct (*ov. d.*), shell gland (*s. g.*), Laurer's canal (*L. c.*), transverse vitello-ducts (*t. vd.*), intestinal ceca (*i.*), vitellaria (*v. g.*), and acetabulum (*ac.*). Enlarged. Original.

FIG. 56.—Optical section. Shows portion of oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*) with granular coagulum, intestinal ceca (*i.*), vesicula seminalis (*v. s.*), arch of union of vasa efferentia (*v. e. a.*), right vas efferens (*v. e. d.*), left vas efferens (*v. e. s.*) passing dorsad from its point of origin on one of the testicular indentations, testes (*t.*), excretory vesicle (*ex. v.*), Laurer's canal (*L. c.*), uterus (*ut.*), acetabulum (*ac.*), and vitellaria (*v. g.*). Enlarged. Original.

CAULIORCHIS,[a] new subgenus.

SUBGENERIC DIAGNOSIS. *Paramphistomum* (p. 73): Ceca distinctly wavy, end in acetabular zone; esophagus without muscular thickening. Acetabulum very large, terminal, tilts ventrad, aperture likely to be rather small. Excretory vesicle not elongate-club shaped, but distended and bag-shaped, its canal rather short, directed dorsad and may extend slightly cephalad or caudad, its pore in vesicular zone. Ventral chamber (?) of genital atrium.

Male organs: Testes very much smaller than acetabulum, cauliflower-like, in pre- or acetabular zones, testicular zones separate, or overlap or coincide, fields separate or overlap or coincide; musculosa well developed, may be enormous, coiled.

Female organs: Ovary ventral to ventro-cephalic of vesicle; Laurer's canal crosses excretory vesicle or canal, its pore is in vesicular zone, caudad to caudo-laterad of excretory pore, the two pores may be quite close to each other.

TYPE SPECIES.—*P. cauliorchis* n. sp.

At least 2 species (*cauliorchis* and *crassum*), probably a third species (*papillosum*), and possibly, but doubtfully, two other species (*indicum* and *calicophorum*) may be placed in this subgenus.

PARAMPHISTOMUM CAULIORCHIS new species.

[Figs. 57 to 70.]

SPECIFIC DIAGNOSIS.—*Paramphistomum* (p. 73): Body 6 to 7.5 mm. long by 3.66 to 4.25 mm. in maximum breadth; grayish buff in color (alcohol specimens); viewed ventrally, pear shaped; viewed laterally, reminds one of an infant's shoe (the caudal end representing the top, the dorsal angle representing the heel, and the oral pole representing the toe); greatest diameter about at junction of equatorial and caudal thirds; tapers rapidly, then gradually to bluntly pointed, rather truncated conical oral pole; caudal end bluntly rounded when viewed ventrally, truncated when viewed laterally; sagittal line of dorsum markedly convex, almost circular at broadest diameter; transverse section nearly circular. Surface with transverse grooves, especially ventrally; oral pole with small conical papillæ; surface folds around aperture of acetabulum. Genital pore ventro-median, about one-fourth of body length from oral pole and at or somewhat caudad of oral sucker and intestinal bifurcation; the pore is situated on large, 0.7 mm., hemispherical, erectile, retractile, bulging structure, the latter encircled by a ridge; it leads into an atrium into which from its dorsal wall, extends an elongate genital papilla, bearing at its vertex the porus hermaphroditicus; the genital papilla is closely encircled by a broad band-like muscular wall of the atrium; atrium divided into a larger dorsal and a smaller ventral portion by a deep circular groove; when the erectile bulging structure is retracted the encircling ridge forms a pair of transverse labia, between which is a transverse slit. Acetabulum anatomically terminal, but because of bending of body of worm it comes to lie ventro-subterminal; sunken into body of worm; very large, 2.5 mm. in dorso-ventral, 3 mm. in transverse diameter, aperture about 0.95 mm., directed ventro-caudad; cavity very deep. Mouth terminal, buccal cavity crateriform, papillated; oral sucker large, 1 to 1.1 mm. long or one-fifth to one-sixth of body length, 0.74 mm. thick, 0.98 mm. broad, pyriform, its papillated lumen broad and flat; sucker lies in a perisuctorial cavity and is bound to parenchyma dorsally and ventrally by mesenterium-like bands; esophagus tortuous, about as long as (at least not shorter than) oral sucker; bifurcation usually cephalad of genital pore; ceca long, extending in wavy course to near caudal margin of acetabulum. Excretory pore dorso-median at or near plane of caudal margin of aperture of acetabulum, 60 to 320μ caudad of Laurer's canal; excretory canal thick walled, arises about on border between third and fourth quarter of length of vesicle; excretory vesicle dorsal of acetabulum, of moderate dimensions.

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[a] From *caulis* (from καυλός), a cabbage stalk, a cabbage [*Brassica oleracea* is the cauliflower], and ὄρχις, testicle.

Male organs: Testes immediately caudad of equator, in same transverse plane, though one may extend very slightly farther cephalad, and farther dorsad also, than the other; irregular in outline, cauliflower-like in section; vas efferens arises from lateral aspect, passes in an arch medio-cephalo-dorsad, then caudad, then medio-dorso-caudad about to plane of origin, then mediad to unite with its fellow to form vas deferens; vas deferens very highly developed, occupying most of intercecal area cephalad of testes; vesicula seminalis intricately coiled; pars musculosa enormously developed, attains 0.4 mm. in thickness; pars prostatica nearly straight, attains 1.2 mm. in length, runs cephalo-ventrad or almost directly ventrad; ductus ejaculatorius rather short, straight, opens into dorsal dilatation of ductus hermaphroditicus cephalad of metraterm. Ductus hermaphroditicus with dorsal dilatation and runs through genital papilla.

Female organs: Ovary considerably smaller than testes, right or left of median line but caudo-mediad of one testis; shell gland smaller than ovary, median and caudad or cephalad of ovary; vitellaria with sparsely scattered small follicles, lateral, extends from about equator of oral sucker about to end of ceca; uterus runs in loops ventrally from shell gland, then turns dorsad, extends cephalad in dorsal portion of median field, bends ventrad under arch of vas deferens, cephalad ventrally of pars musculosa to open into dilated portion of ductus hermaphroditicus; Laurer's canal runs from oviduct, crosses on right or left of excretory vesicle, and opens slightly to right or left of median line, 60 to 320μ cephalad of excretory pore.

Eggs: Not observed.

TYPE: U.S.N.M. 7155. Cotype U.S.B.A.I. 15026.

HABITAT.—In (organ? of) *Bos indicus*, Sanawaar, Punjab, India.

SOURCE OF MATERIAL.—Four specimens were found in a bottle bearing the U.S.N.M. number 5775 and three in a bottle bearing B.A.I. number 1723. These were renumbered U.S.N.M. 7155 and 15026, respectively. Aside from the difference in the numbers the labels in the two bottles bear the following legend:

> "Name *Amphistoma crumeniferum*. Locality, Sanawaar, Punjab, India. Host, *Bos indicus*. Collected by Dr. Giles. Date, III, 1893. Determined by Dr. Giles. Date, 1893. Presented by Dr. Giles. Date, 1893."

EXTERNAL CHARACTERS.

SIZE.—The 7 specimens forming the collection vary from 6 to 7.5 mm. in length. The greatest width varies from 3.66 to 4.25 mm. in 3 of the 7 specimens in which this diameter was measured.

COLOR.—The worms were of a grayish buff tint.

FORM.—The form is typically shown in figs. 57–60. In transverse section the body of the worm is approximately circular and its greatest transverse and dorso-ventral diameters are at about the junction of the equatorial with the caudal third of the body. From this region of the body these diameters become gradually reduced in the direction of both poles; toward the oral pole the reduction is rapid for a short distance, then it becomes very slow, the total reduction being considerable: while toward the aboral pole it progresses uniformly, but the total is slight. In consequence of this the worm is bluntly pointed and the cephalic portion of the body appears somewhat cylindric and distinctly attenuated as contrasted with the massive ventrally bent caudal portion.

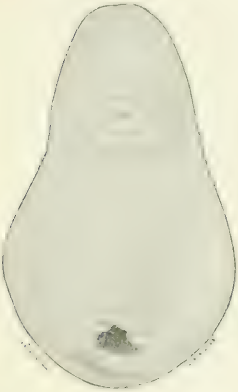


FIG. 57.

SURFACE.—The surface cuticle is unarmed; that is, it is devoid of scales or spines, but at the oral pole close to the aperture of the mouth the surface is provided with small rounded and conical papillæ. It is also marked by transverse grooves, which are best defined on the venter. At the caudal extremity around the aperture of the acetabulum the worm is deeply wrinkled, forming rounded folds and tabs which encroach upon and apparently reduce the size of the acetabular aperture (figs. 57, 60).

Genital pore.—In the median line of the ventral surface at about the junction of the first with the second fourth of the body is the genital pore (figs. 57 59, 61 63, 67). This pore, which measured 0.08 mm. in diameter in one specimen, is at the vertex of a hemispherical bulging which measured about 0.7 mm. in diameter. Encircling the base of this bulging structure is a ridge in the form of a transverse ellipse. The genital pore leads into a cylindrical dorso-ventrally running atrium, from the dorsal wall of which arises a somewhat elongate trumpet-shaped genital papilla. This papilla projects ventrad into the atrium for about two-thirds the length of the latter and is closely embraced by the encircling muscular ring-like atrium wall (figs. 62, 67). The atrium wall is divided into two unequal rings by a deep narrow circular groove; the larger or dorsal of the rings forms about two-thirds of the atrium wall and is the portion which has just been described as closely embracing the genital papilla. In section it is seen that these rings differ markedly in structure, the larger or dorsal ring being of a dense muscular structure, whereas the structure of the smaller ring is loose and but slightly muscular.

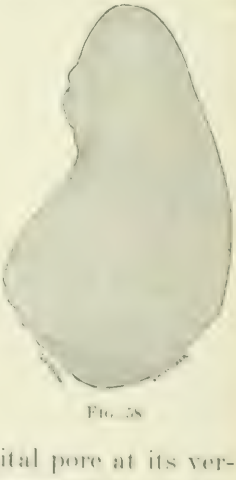


FIG. 58.

The hemispherical bulging which bears the genital pore at its vertex, is of an erectile nature, and the worm has the power of causing its retraction. In the retracted state the ridge which has been described as forming an encircling ellipse about the base of the hemispherical bulging, forms two transverse lips continuous laterally (figs. 59, 63).

Between these lips is a transverse more or less slit-like aperture which gives entrance to an irregular dorso-caudally directed passage which leads directly to the hermaphroditic pore.

Acetabulum.—The acetabulum is in the caudal terminal portion of the worm, but on account of the more or less decided bending ventrad of this portion of the body the acetabular aperture is tilted in the same direction. In one of the sectioned specimens this muscular organ, with an aperture measuring about 0.95 mm. in diameter, measured 2.5 mm. in dorso-ventral, 3 mm. in transverse diameter.

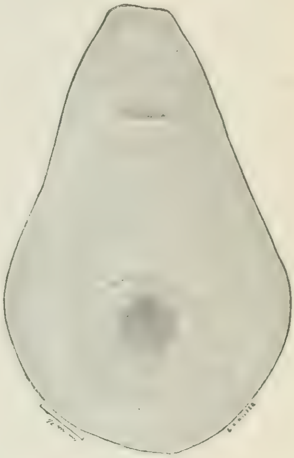


FIG. 59.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The bluntly pointed cephalic extremity presents a circular crateriform depression which is beset by conical papillæ; it leads into the oral sucker through a small circular opening which may be designated its oral aperture. The sucker is large and muscular, varying in length between 1.08 and 1.18 mm., or from one-fifth to one-sixth the body length as measured in sections of 5 specimens. In one of these (figs. 61, 62) the extreme dorso-ventral and transverse diameters were 0.74 mm. and 0.98 mm., respectively; its form in median sagittal plane is shown in figure 62. It will be noticed that it resembles the retracted pear-shaped sucker of *P. calicophorum*, as described and pictured by Fischœder (1893h, p. 542, fig. 30). The sucker as a whole may, for purpose of description, be regarded as consisting of a dorsal and a ventral muscle, continuous one with the other laterally. These two muscles are each thickest in the median sagittal plane; they grow less thick as they extend laterad, being thinnest laterally where they are continuous one with the other. The opposing faces of these muscles are closely approximated, so that the lumen of the sucker is reduced to a dorso-ventrally very narrow slit-like space; the latter is lined with a cuticle-like layer in anatomical continuity with that of the surface

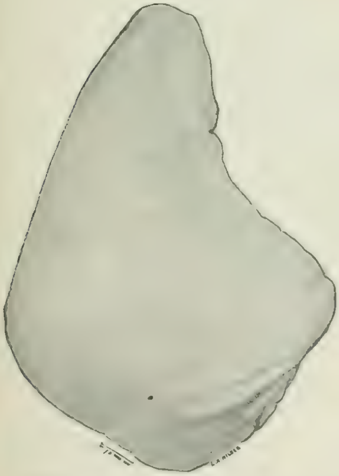


FIG. 60.

and is beset by very small closely aggregated papillæ. These papillæ are largest and most prominent in about the equatorial region of the sucker, becoming gradually smaller and less distinct as the oral and esophageal apertures are approached, close to both of which these papillæ do not appear to be present. Except at the oral and the esophageal ends the sucker is separated from the body parenchyma by a distinct circumsuctorial space, strongly suggestive of a body cavity, which is traversed here and there by mesenterium-like strands attaching the sucker, particularly the dorsal and ventral walls, to the inclosing parenchyma. The space contains, but does not appear to be at all filled with, a granular material and a few nuclei (?), the cell bodies of which are not clearly definable.

Dorsally of the circumsuctorial cavity and on a plane near the caudal end of the sucker is found the brain.

The esophagus takes origin at the base of the sucker. At a point about midway between its point of origin and the dorsal wall of the worm and at about the same or slightly lower (more caudad) level, namely, at from two-sevenths to one-fourth the body length from the oral margin, the esophagus divides into two intestinal ceca. The path pursued by the esophagus between its point of origin and its bifurcation was found to vary according to two general types; in one (figs. 61, 62, 64), to which 3 of the 5 sectioned specimens conformed, the esophagus almost immediately after leaving its point of origin tends ventrad and to either the left (2 specimens) or the right (1 specimen), curving around and skirting more or less closely the base of the sucker until it gains either the left or right dorso-lateral aspect of the latter, beyond which it proceeds in a more direct course to its point of bifurcation. In the second type (fig. 63), to which 2 of the 5 specimens conformed, the esophagus passes from its point of origin in a direction caudad and at the same time more or less ventrad for a variable distance. It then abruptly doubles back on itself, describing a U-shaped course in about the median sagittal plane of the body. The dorsal limb of the U quickly assumes a more or less direct course dorsad to the point of its bifurcation.

From their point of origin the intestinal ceca pass at first almost horizontally laterad, then curve gently latero-caudad and slightly ventrad to reach a point about 0.33 mm. from the body wall. Occasionally one or both ceca may arch slightly cephalad of their point of origin as they pass laterad. The ceca terminate at slightly different levels, but close to the dorso-lateral aspect of the acetabulum and a little above the plane of the caudal margin of the latter. In their path caudad the ceca describe a wavy course approximately parallel to the dorso-lateral aspect of the body wall (figs. 61, 62).

The lumen of the esophagus is lined with a continuation of the cuticle-like layer of the sucker. The intestines are lined with a

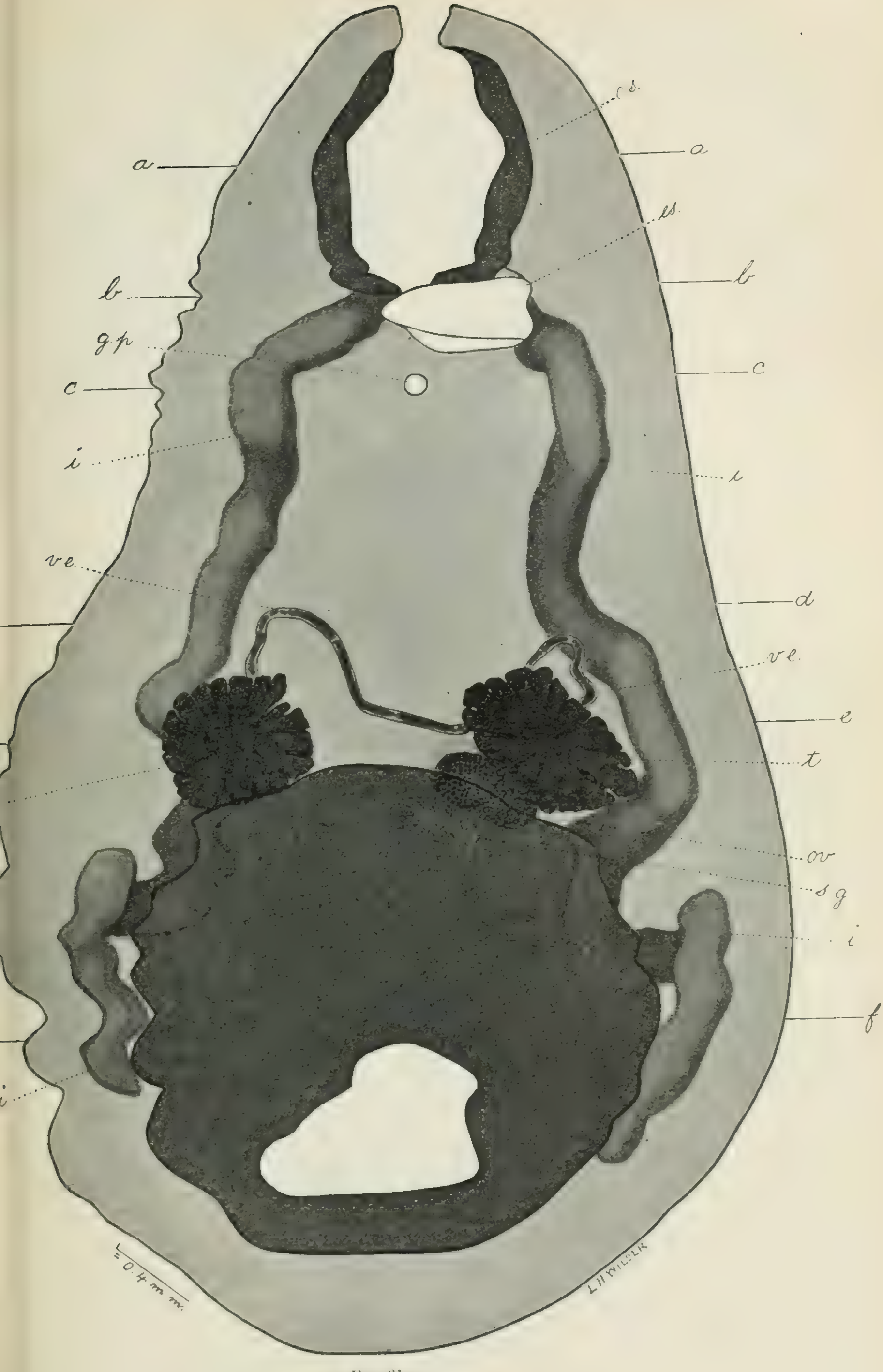


FIG. 61.

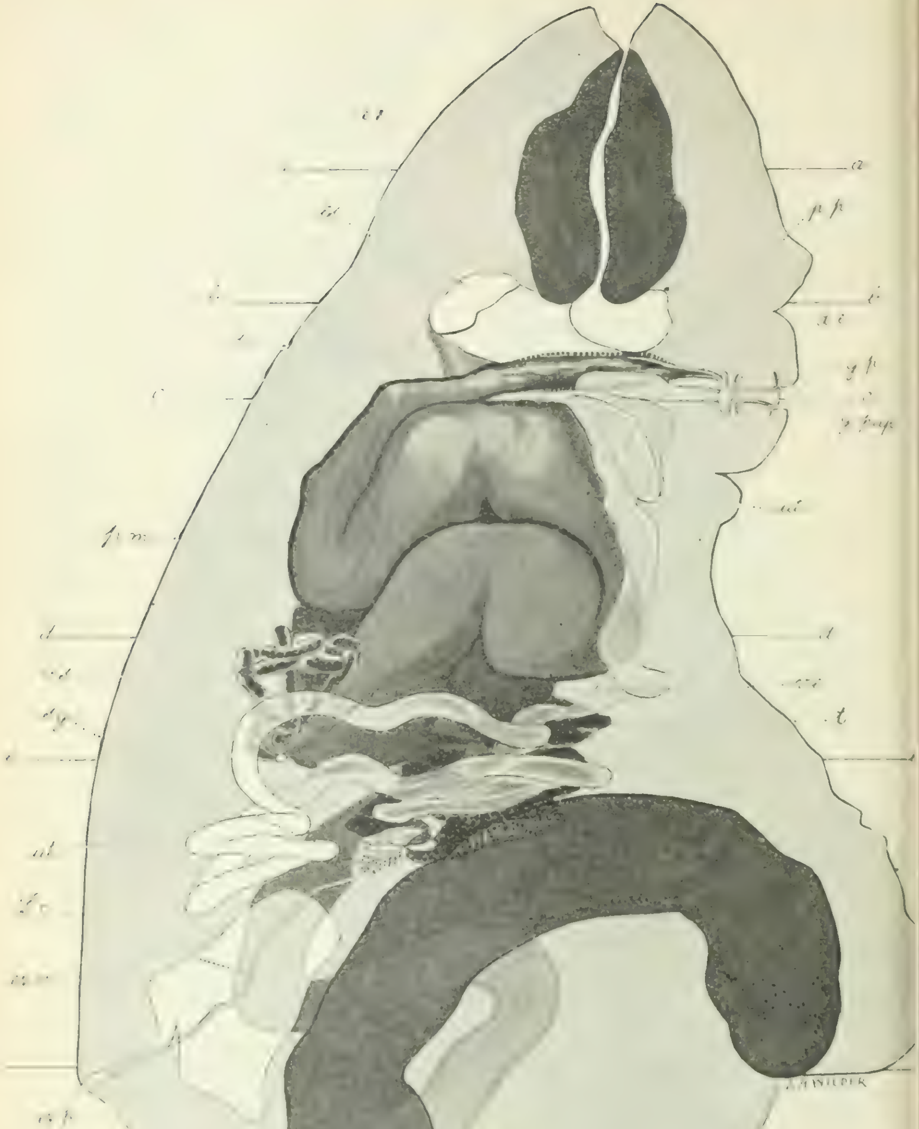


FIG. 62.

layer of epithelium, which begins abruptly at the bifurcation of the esophagus.

GENITAL ORGANS.—Excepting the vitellaria, both the male and the female genital organs are situated in the intercecal area.

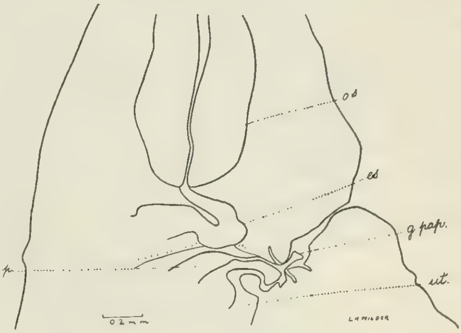


FIG. 63.

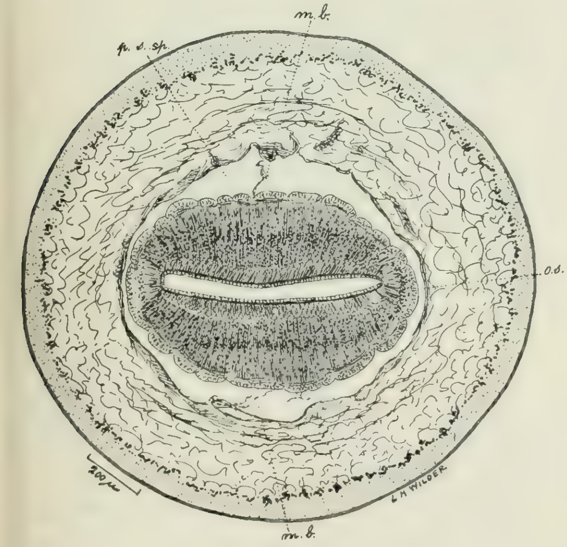


FIG. 64.

Male organs.—The testes are in the zone immediately caudad of the equatorial plane, one on either side of the median sagittal plane. Further than this, however, the relation which they bear one to the other and to the acetabulum is subject to marked variation. Their superior (cephalic) margins may be in the same (3 specimens) or in different (2 specimens) transverse planes; in 2 specimens in which the latter condition obtained the superior margin of the left testis was 0.2 mm. cephalad of that of the right in one case, while in the other it was the superior margin of the right testis that was cephalad of that of the left, and in this instance the difference was 0.26 mm. Furthermore, in each of these 2 specimens the higher (cephalad) testis was also the farther dorsad. In 2 of the 3 specimens in which the superior margins of the testes were found in the same transverse plane the right testis was a little farther dorsad than the left; in the third of these specimens both testes were in about the same frontal plane. It may be observed in this connection that a certain relation appears to exist between the position of the testes and that of the ovary and shell gland, namely, in 4 of the 5 specimens in which one of the testes was placed farther dorsad than the other the ovary and shell gland were found on that side of the median sagittal plane to which the dorsally placed testis belonged.

In 2 of the specimens the testes were immediately cephalad of the superior margin of the acetabulum; in 2 others they were immediately caudad of this level, and in a fifth they were at this level. They are greatly branched, cauliflower-like in sections, and differ slightly in size in the same and in different specimens. Measurements, from projections, of the left testis in one of these worms gave 1.2 mm. for the greatest dorso-ventral, 0.6 mm. for the greatest transverse, and 0.64 mm. for the greatest longitudinal diameter. A vas efferens arises from the external lateral aspect of each testis; these unite to form the vas deferens. The point of union of the vasa efferentia is generally at about the same level as the point of origin of one or both the vasa efferentia and more or less dorsad of the mid point in the ventro-dorsal diameter. The path pursued between their point of origin and their union is peculiar and differs from that in any of the other forms studied. Each vas at first passes medio-cephalo-dorsad for a variable distance, in some of the specimens considerably above the superior margin of the testis (figs. 61, 62) from which one or both originates. Each then turns caudad, describing a more or less well-marked curve, with its convexity cephalad, and then proceeds medio-dorso-caudad to about the level at which one or both originated, where they pass more or less directly inward to unite, as

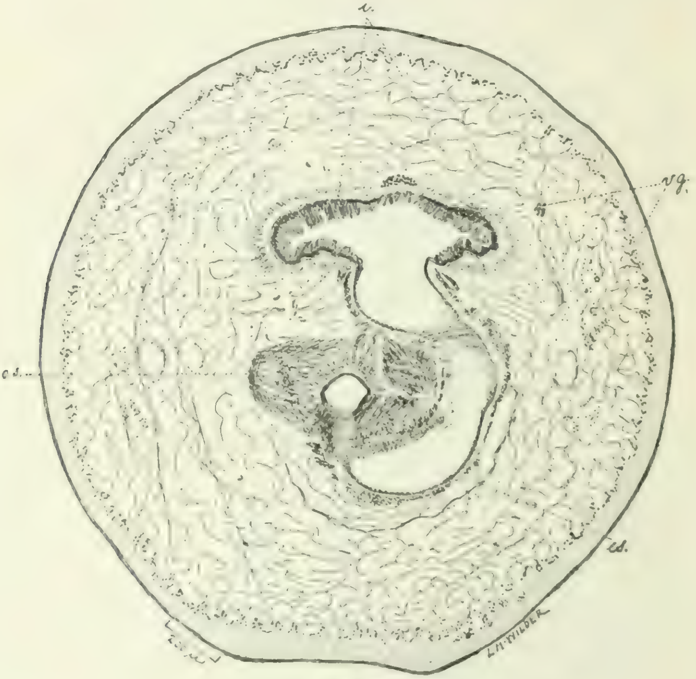


Fig 65.

has already been said, in the formation of the vas deferens. The vasa are fairly thick-walled ducts, measuring 60 to 75 μ in diameter in one of the specimens.

The vas deferens is very highly developed and occupies most of the space in the axial region of the body between the intestinal ceca cephalad of the testes. As in the other forms of this group, it is clearly differentiated into four portions. The first portion, or vesicula, is an intricately coiled, thin-walled, and but slightly dilated duct, which is succeeded by a strikingly large, thick, muscular-walled second portion, or pars musculosa, which measured 0.4 mm. in diameter, with a thickness of wall of 0.15 mm. in one specimen. The change from vesicula to musculosa is abrupt and is marked not only by the enormous increase in the diameter of the duct and in the thickness of its wall, but by the interposition of a short section, which from its arrangement probably serves the purpose of a valve. This valvular segment—or pars intermedia (*p. i.*, fig. 68), as it may be designated—is a direct continuation of the vesicula, but the caliber of its lumen is greatly reduced, its walls are thick and muscular, but its total diameter is little, if at all, greater than that of the vesicula. Its distal end pierces the enormously thick muscular wall of the pars musculosa, into the lumen of which it projects in a manner resembling the projection of the cervical portion of the uterus into the human vagina. The musculosa is compactly coiled; the windings run in a vertical direction and, unlike those of the vesicula, can be traced without much difficulty. The musculosa is succeeded by the pars prostatica; the change from one to the other is marked by a structure suggesting

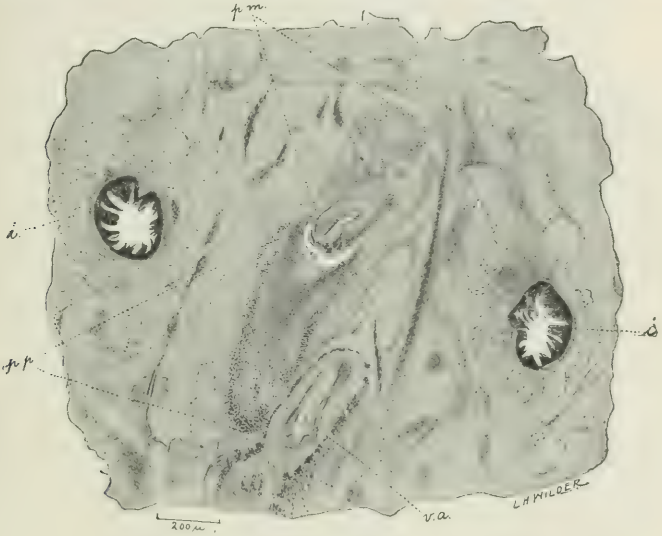


Fig. 66.

a valvular function (fig. 67). Except that there is not interposed an intermediate section between the musculosa and the prostatica, the valve-like arrangement appears to be exactly like that at the junction of vesicula and musculosa, with the further difference that the former is considerably smaller in diameter. As this point is approached, the musculosa becomes gradually reduced in diameter and its wall in thickness. The prostatica is not coiled; it pursues an almost straight or slightly sinuous course ventrad or cephalo-ventrad. In the latter case it describes a slight curve in a sagittal plane, with the convexity of the curve cephalad. Throughout its length it is inclosed in a mass of cells; in projection of one specimen the prostatica measured 1.2 mm. in length.

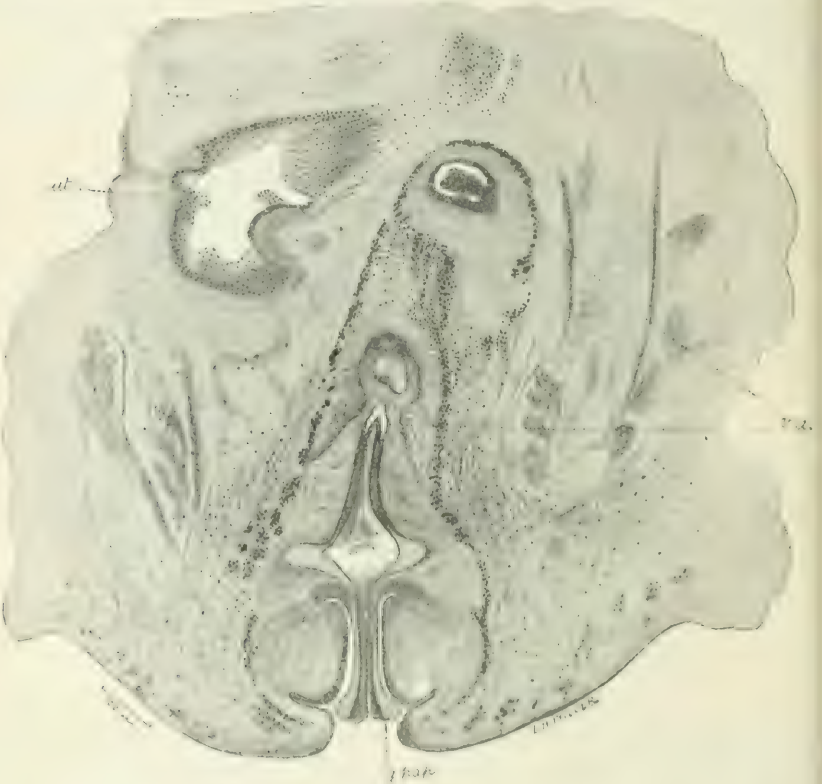


Fig. 67.

The diameter of this portion of the vas deferens becomes gradually reduced in its course ventrad. The reduction is for the most part at the expense of the lumen, for the walls remain highly muscular and but slightly reduced in thickness. The pars prostatica is succeeded by the fourth or terminal portion of the vas deferens. This portion, or ductus ejaculatorius, is a direct continuation of the prostatica, the change from one to the other being defined only by the cessation of the cell mass characteristic of the prostatica. It passes directly ventrad and opens by a minute pore into the dorsal dilated vesicle-like portion of the ductus hermaphroditicus immediately above and quite separate from the aperture of the metraterm (fig. 62). It measures about 0.24 mm. in length in one specimen. The ductus hermaphroditicus is a delicate duct which pierces the axial region of the genital papilla to open externally as the porus hermaphroditicus. It leads from an atrium or vesicle which may perhaps be regarded as the dilated internal extremity of the duct. Into this vesicle or atrium there open the ductus ejaculatorius and the metraterm.

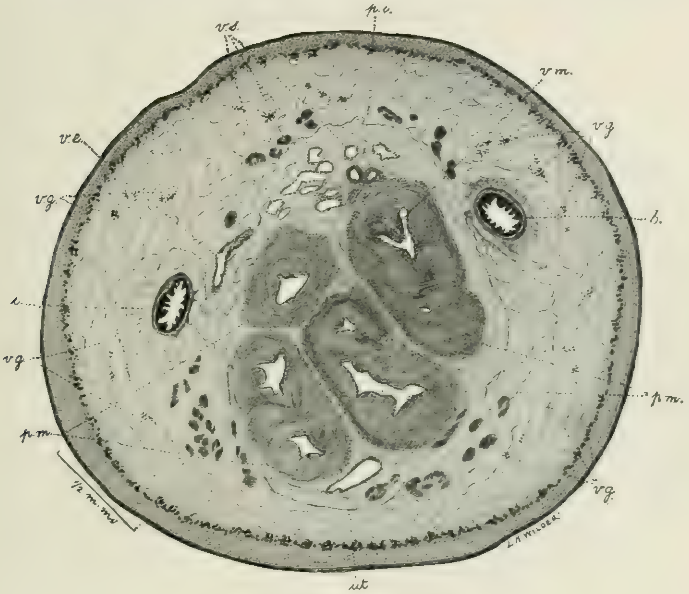


FIG. 68.

Female organs.—The ovary varies somewhat in size, form, and position, and in its relation to the shell gland. It is considerably smaller than either testis, close to one of which it is placed. In 3 of 5 specimens studied the ovary was on the right side of the median line close to the ventro-mesio-caudal aspect of the right testis; in the other 2 specimens it was on the left side, in one slightly caudad of the testis and in the other close to the ventro-mesio-caudal aspect of the left testis. The shell gland is smaller than the ovary but like the latter varies somewhat in size, form, and position; in 4 of 5 specimens it was close to the mesio-dorso-caudal aspect of the ovary; in the fifth specimen it lay close to the mesio-cephalic aspect of the ovary. The oviduct springs from the dorsal pole of the ovary and passes to the shell gland which it penetrates at some point on its dorsal aspect, describing in its more or less horizontal course a well-marked curve with its convexity dorsad.

Laurer's canal leaves the oviduct from some point more or less close to the shell gland and opens on the dorsum by a minute pore slightly to the right (twice) or to the left (fig. 70) (three times) of the median line and from about 60μ to 320μ cephalad of the excretory pore. It will be remembered that in the 5 specimens studied the ovary was found on the right side in 3; in 1 only of these 3 specimens did Laurer's canal open to the right of the median line, in the other 2 it opened to the left of this line. In the 2 of the 5 specimens in which the ovary was found on the left side, Laurer's canal opened on the left side in one and to the right in the other. It may therefore be easily understood that the path described by Laurer's canal in its course to the dorsal opening varies widely. In general it may be said that this canal, after leaving the oviduct, passes diagonally to the other side of the median line over the excretory vesicle to gain the dorsal aspect of the latter, then proceeds dorso-caudad to the opening on the dorsal surface. In the minority of cases (2 in 5) the canal remains on the side (either right or left) in which it takes origin, skirts the lateral margin of the excretory vesicle as it proceeds dorso-caudad, gains the dorsal aspect of the vesicle and opens on the dorsal surface on the same side of the median line with its point of origin.

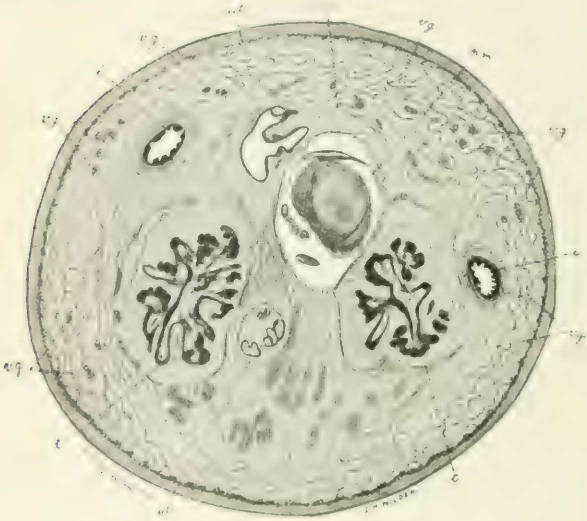


FIG. 69.

The vitellaria, consisting of sparsely scattered insignificant follicles, occupy the lateral fields of the body external to the intestinal ceca. They extend longitudinally from about the level of the equator of the sucker to about the level of the cecal extremities of the intestines. A duct leaves each gland at a point a little caudad of the level of the superior margin of the acetabulum and passes transversely inward with a more or less marked tilt cephalad, ventrally of the corresponding testis, to unite with its fellow close to the caudo-ventral aspect of the shell gland. From their point of union a duct passes dorsad skirting the caudal or one of the caudo-lateral margins of the shell gland to penetrate the latter at some point at its caudo-dorsal aspect.

Within the shell gland this vitello-duct joins with the oviduct to form a common canal which almost immediately forms a fusiform ootype. The direction of the ootype is ventrad or ventro-cephalad, sometimes with a slight tilt to either the right or left. The ootype is continued as the uterus which emerges at some point on the ventral or ventro-lateral aspect of the shell gland. After emerging the uterus forms some coils ventrad or ventro-mediad of the shell gland; it then winds its way dorsad over the excretory vesicle or skirts one side of the superior portion of the latter. Dorsad of the excretory vesicle several loops dip caudad; it then winds its way cephalad in the dorsal part of the median field, then bends ventrad winding its way beneath the arch of the vasa efferentia close to the caudal aspect of the coiled pars musculosa to reach the ventral aspect of the latter; having reached this point the uterus bends abruptly cephalad and with but few windings maintains this relation to the vas deferens throughout the remainder of its course, opening, as has already been mentioned, into the dilated vesicle-like portion of the ductus hermaphroditicus immediately beneath the opening of the ductus ejaculatorius.

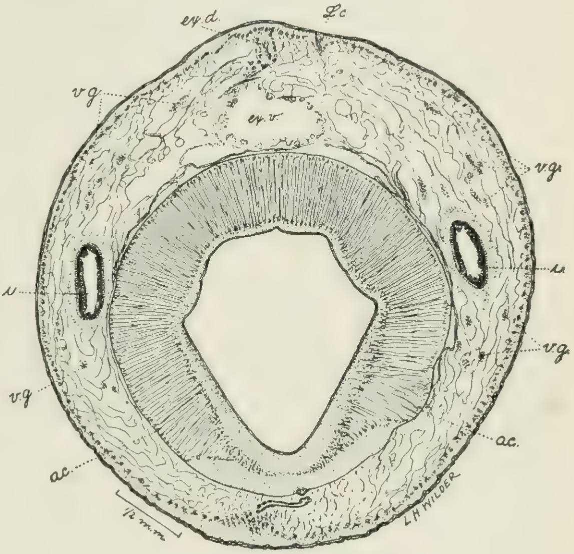


FIG. 70.

Eggs.—Eggs were not observed in any of the specimens.

EXCRETORY SYSTEM.—The excretory system is well developed. A vesicle of moderate dimensions lies dorsally of the acetabulum. From the dorsal aspect of this vesicle from a point which varies considerably in position, but which may be described as at about the junction of the third with the fourth quarter of the length of the vesicle, there arises a moderately thick excretory duct. This duct of somewhat variable length passes at a variable angle caudo-dorsad to open in the median line of the dorsal surface at or somewhat caudad of the level of the caudal margin of the aperture of the acetabulum, and, as has been described, from 60μ to 320μ caudad of the aperture of Laurer's canal.

ILLUSTRATIONS.

Fig. 57.—Ventral aspect of *P. cauliorchis*, copulatory apparatus evaginated. Enlarged. Original.

Fig. 58.—Profile of same.

Fig. 59.—Ventral aspect, copulatory apparatus invaginated. Enlarged. Original.

Fig. 60.—Profile of same.

Fig. 61.—Ventral projection of *P. cauliorchis* shown in figs. 57 and 58. *g. p.*, position of genital pore; *es.*, esophagus; *i.*, intestinal ceca; *o. s.*, oral sucker; *ov.*, ovary; *s. g.*, shell gland; *t.*, testes; *v. e.*, vasa efferentia. Uterus and vas deferens not shown; *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f* planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 62.—Profile projection of *P. cauliorchis* shown in figs. 57 and 58. *ac.*, acetabulum; *g. p.*, genital pore; *d. e.*, ductus ejaculatorius; *es.*, esophagus; *ex. p.*, excretory pore; *ex. v.*, excretory vesicle; *g. pap.*, genital papilla; *i.*, intestinal ceca; *L. c.*, Laurer's canal; *o. s.*, oral sucker; *ov.*, ovary; *p. m.*, pars musculosa; *p. p.*, pars prostatica; *s. g.*, shell gland; *t.*, left testis; *ut.*, uterus; *v. e.*, left vas efferens; *v. s.*, vesicula seminalis; *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f* planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 63.—Profile projection showing genital papilla retracted and lips of genital aperture slightly ajar. Lettering as in fig. 62. Slightly diagrammatic. Enlarged. Original.

Fig. 64.—Transverse section at *a–a*, figs. 61 and 62. Shows oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), mesenterium-like strands (*m. b.*). Enlarged. Original.

Fig. 65,—Transverse section at *b–b*, figs. 61 and 62. Shows base of oral sucker (*o. s.*), the esophagus (*es.*), origin of intestinal ceca (*i.*), and some vitelline follicles (*v. g.*). Enlarged. Original.

Fig. 66.—Portion of a transverse section to show valve at junction of pars musculosa (*p. m.*) and pars prostatic (*p. p.*).; *i.*, intestinal ceca; *va.*, metraterm. Enlarged. Original.

Fig. 67.—Portion of a transverse section at *c–c*, figs. 61 and 62, to show evaginated copulatory apparatus. *g. pap.*, genital papilla; *va.*, metraterm; uterus (*ut.*). Enlarged. Original.

Fig. 68.—Transverse section at *d–d*, figs. 61 and 62. Shows pars musculosa (*p. m.*), vesicula seminalis (*v. s.*), pars intermedia (*p. i.*), valve at junction of vesicula and musculosa (*v. m.*), uterus (*ut.*), right vas efferens (*v. e.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 69.—Transverse section at *e–e*, figs. 61 and 62. Shows position and relations of the cauliflower-like testes (*t.*), uterus (*ut.*). Caudal margin of pars musculosa (*p. m.*), arch of union of the vasa efferentia (*v. e.*), the intestinal ceca (*i.*), and the vitellaria (*v. g.*). Enlarged. Original.

FIG. 70.—Transverse section at *f–f*, figs. 5 and 6. Shows acetabulum (*a c.*), excretory vesicle (*ex. v.*), excretory duct (*ex. d.*), Laurer's canal (*L. c.*), vitellaria (*v. g.*), and intestines (*i.*). Enlarged. Original.

PARAMPHISTOMUM CRASSUM, new species.

[Figs. 71 to 80.]

SPECIFIC DIAGNOSIS.—*Paramphistomum* (p. 73): Body 6.12 to 6.5 mm. long by 4.2 to 4.76 mm. broad; color drab (alcohol material): in ventral view broad pyriform, greatest transverse diameter at junction of equatorial and caudal thirds; tapers gradually cephalad to plane of genital pore, then abruptly, then gradually to blunt oral pole; caudad it tapers rapidly, so that caudal margin is very bluntly rounded; longitudinal axis curved, convexity dorsad; in transverse section transversely elliptical. Surface with transverse grooves, more marked on venter; cephalic cone with acne-like papillæ, which become more acuminate and slender around oral aperture. Genital pore ventro-median in suctorial zone at junction of oral with second fifth of body; at this point there is a slight ventral elevation. Acetabulum large, ventral, 3.1 mm. in transverse, by 2.74 mm. in dorso-ventral diameter, sunken into body, cavity deep, aperture very small, 0.5 mm. in transverse diameter, directed slightly ventrad. Mouth subterminal; oral sucker very large, 1.34 mm. long, 1.26 mm. broad, 0.98 mm. thick, its lumen broad and shallow, papillated; perisuctorial space narrow; esophagus shorter than sucker, bent U-shaped, convexity ventrad; ceca run at first latero-cephalad, then in wavy to zigzag course to zone of acetabular aperture, then turn slightly cephalad and end about at equator of acetabulum. Excretory pore opens at postovarial plane; canal short, thick; vesicle large, dorsal of acetabulum, extends from preovarian plane into zone of acetabular aperture, and is crossed by Laurer's canal.

Male organs.—Testes large, cauliflower like, in intercecal area, diagonal, zones overlap, fields overlap; vasa efferentia arise from lateral aspect, run cephalad, then dorso-mediad, uniting slightly postequatorial, in zone of anterior testis; vesicula seminalis loosely coiled; musculosa complexly and compactly coiled, connected with vesicularis by a pars intermedia; prostatica nearly straight; ductus ejaculatorius pierces a well-developed muscle mass and opens with metraterm into a slit-like space at base of minute genital papilla, and continued as ductus ejaculatorius, which opens at apex of genital papilla.

Female organs.—Ovary dextral of median line, in acetabular zone, partly in testicular zone, in field of anterior testis, smaller than testis; shell gland dorso-caudad of ovary and nearer median line; vitellaria consist of sparsely scattered follicles, extending from plane of genital pore to slightly postcecal; uterus extends in coils from shell gland cephalad, turns caudad ventrally of left testis, cephalad dorsally of same, under arch of vasa efferentia, ventrally of vesicle to the same slit-like space into which the ejaculatorius discharges; Laurer's canal extends from oviduct dorso-caudad, crosses excretory vesicle and opens submedian, about 0.7 mm. caudad of excretory pore.

Eggs.—Not observed.

TYPE.—U.S.N.M. 7156 (Coll. Hassall).

HABITAT.—In (organ ? of) *Bos indicus*, India.

Source of material.—The material consisted of 3 specimens found with other forms in bottle 5775, collected by Doctor Giles in 1893 in India (Sanawaar, Punjab), from *Bos indicus*. These specimens were given the number U.S.N.M. 7156.

EXTERNAL CHARACTERS.

Size.—Two of the specimens, measured in glycerin alcohol, were 6.5 mm. long; the third specimen measured in sections was 6.12 mm. long. The greatest transverse diameter of one of the specimens, measured in glycerin alcohol, was 4.20 mm., while that of another specimen measured in sections was 4.76 mm.

Color.—The worms are of a drab color.

Form.—The form of these worms is shown in fig. 71. They somewhat resemble *Paramphist. cauliorchis*. The greatest transverse diameter is at about the junction of the equatorial with the caudal third of the body length. From this region, in the direction of both poles, the breadth of the animal becomes progressively reduced, rapidly toward the caudal pole, making this extremity broad and rounded, more gradually toward the oral pole until the level of the genital pore is reached. Here there is a rather abrupt reduction in the transverse diameter, clearly shown in fig. 71. Beyond this point the progressive reduction in breadth is very gradual, so that the lateral margins of this oral portion of the worm are very nearly straight lines, whereas the other portions of the lateral margins of the worm are decidedly curved. The dorso-ventral diameter is relatively shorter in this form than in *Paramphist. cauliorchis*, so that when viewed ventrally it gives the impression of being more decidedly flattened. Viewed in profile it is seen that the longitudinal axis of the worm is curved with the concavity of the curve ventrad. The outline of the worm in transverse section is transversely elliptical.

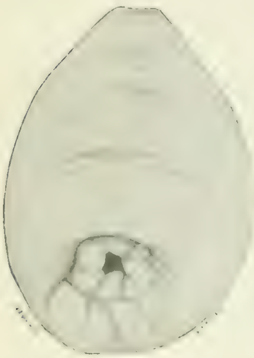


Fig. 71.

Surface.—The surface cuticle is without spines or hooks, but is marked by transverse grooves, which are best defined and deepest on the ventral surface. The cephalic cone-like portion is beset by acne-like papillæ. In the region immediately around the oral aperture the papillæ become somewhat acuminate and appear slender and somewhat more raised above the general cuticular surface.

Genital pore.—In the median line of the ventral surface, at about the junction of the oral with the second fifth of the body, there is a transverse buttonhole-like slit, measuring in one alcohol specimen

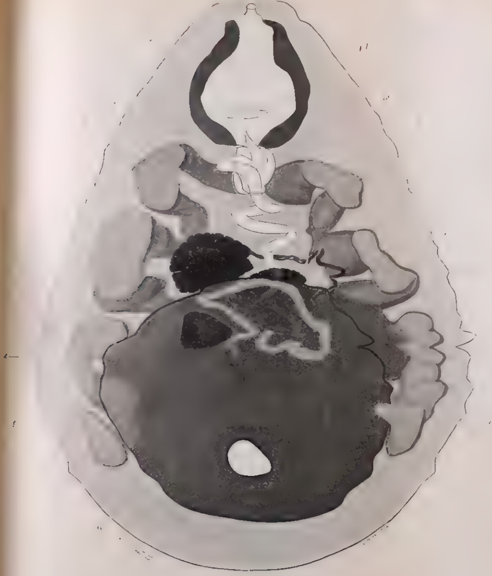


FIG. 72

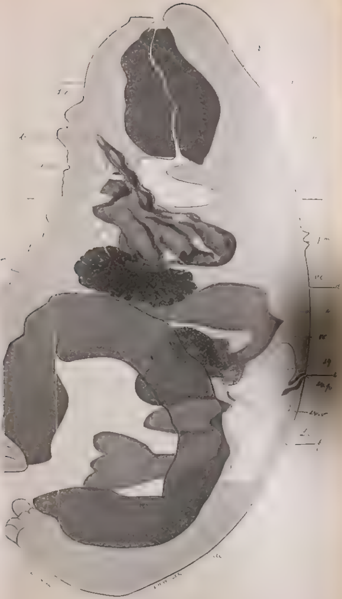


FIG. 73

about 0.45 mm. in length. This slit, or slit-like depression, is at the vertex of a moderate, transversely elongate elevation, and represents the genital pore.

Acetabulum.—The acetabulum is large, and, as in the other forms of this group, it is in the caudal portion of the body. Its relatively small aperture is directed slightly ventrad on account of the curve of the body. Measurements taken from the projection of one sectioned specimen give 2.74 mm. as the greatest dorso-ventral and 3.10 mm. as the greatest transverse diameter of the acetabulum, with 0.34 mm. as the greatest verticle and about 0.50 mm. as the greatest transverse diameter of the acetabular aperture. The true aperture of the acetabulum is somewhat reduced in size by being encroached upon by folds of the adjacent body surface, much as in the case of *Paramphist. cauliorchis.*

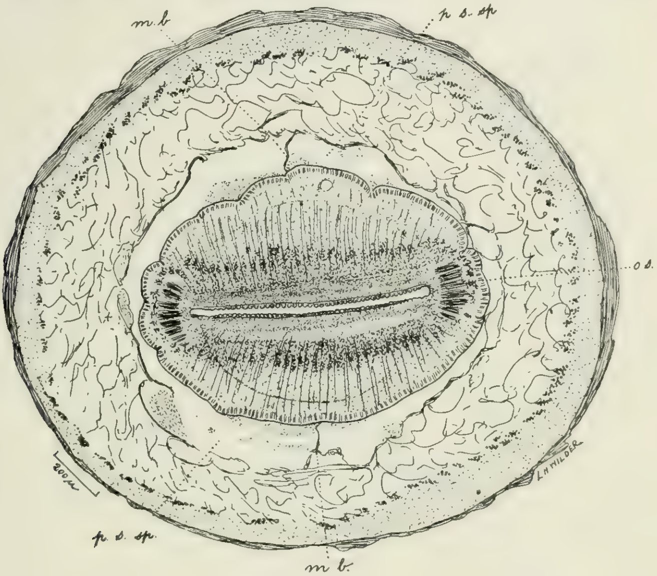


FIG. 74.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The cephalic pole is marked by an irregularly circular aperture, measuring in section 90μ by 120μ in diameter. This aperture leads into a shallow irregular cavity which gives entrance to the oral sucker. The latter is a large muscular organ, which in projection of one specimen measured 1.34 mm. in longitudinal axis, 0.98 mm. in greatest dorso-ventral diameter, and 1.26 mm. in greatest transverse diameter. The dorsal and ventral walls are thick, measuring 0.46 mm. and 0.50 mm. in thickness, respectively, when measured in the greatest dorso-ventral diameter of the sagittal plane. Laterally these muscular plates are thinner and continuous one with the other. The organ is inclosed in a narrow space in the body parenchyma (figs. 74, 75), in which it is retained in position by attachments at its two poles and by dorsal and ventral mesenterium-like strands. The lumen of the sucker is a dorso-ventrally, very narrow, but transversely quite broad, cavity. In transverse sections it appears as a transverse slit lined by a thin cuticle-like layer, which is beset by short conical papillæ; the latter are largest in the oral half of the lumen, become gradually smaller and more slender in the direction of the esophageal end, near which they disappear altogether. The esophagus springs from the caudal or basal aspect of the sucker, then describes a more or less well-marked U-shaped course with the base of the U ventrad. The caudal limb of the U is much the longer and is directed almost horizontally dorsad, having only a slight tilt caudad; it divides into the 2 intestinal ceca. These at first pass laterad with a tilt cephalad to a plane slightly above their plane of origin, describing at the same time a slight curve having its convexity dorsad. After approaching the ventro-lateral aspect of the body wall, each of the intestinal tubes bends caudad and describes a decidedly wavy, almost zigzag, course caudad, approximately parallel to the lateral body wall of the worm. The cecal extremities are directed ventrad close to the sides of the acetabulum and at about the level of the upper margin of the acetabular aperture.

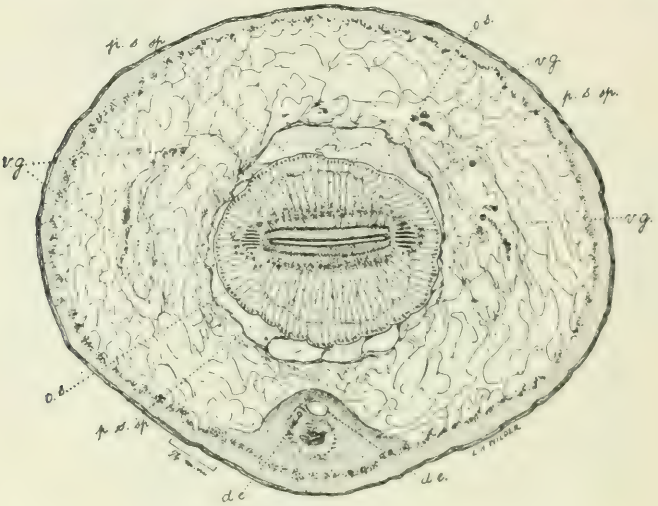


FIG. 75.

The lumen of the esophagus is lined by a cuticle-like layer continuous with that of the oral sucker, but terminating abruptly at the point of origin of the ceca. The latter are lined by an epithelial cell layer.

Genital system.—With the exception of the vitellaria, the genital organs are disposed in the intercecal space.

Male organs.—The testes are in the equatorial zone of the worm, one laterad of the other, and separated by a moderate interspace. They are in separate but more or less overlapping testicular zones and fields (fig. 72). In both of two sectioned specimens the right testis was the more cephalad. In transverse sections the testes appear to be composed of numerous lobules suggesting a cauliflower-like appearance (fig. 78). From the external aspect of each testis there emerges a vas efferens, which tends at first cephalad, then dorsomediad, finally uniting with its fellow to form the vas deferens (fig. 72). The vas deferens is distinctly divisible into a vesicula, musculosa, prostatica, and ductus ejaculatorius. The vesicula is loosely but complexly coiled, thin walled, and but slightly dilated. It is succeeded by a complexly and somewhat more compactly coiled musculosa. The muscular walls of this portion of the vas deferens are well developed, but not nearly so much as in *Paramphist. cauliorchis;* measured at a favorable point the diameter of the duct was 0.24 mm., with a thickness of wall of 0.067 mm. There is in this, as in *Paramphist. cauliorchis*, a short intermediate segment or pars intermedia between the vesicula and musculosa. This segment is muscular but of less diameter and both much thinner walled and of smaller caliber than the musculosa. The change from musculosa to prostatica is clearly marked by a change in the structure of the wall and by the appearance of a thin layer of cells which inclose this third portion of the vas deferens. The prostatica is not coiled, but pursues a direct course cephaloventrad. As it nears a prominent somewhat globular muscular mass, which incloses the terminal genital (copulatory) apparatus, the prostatic cells are lost, and the prostatica now continues as the ductus ejaculatorius. It penetrates this muscular mass in close relation to the dorsal aspect of the terminal portion of the uterus. This portion of the male duct is still of considerable caliber and thin walled, giving the impression of a vesicle, but after a very short course becomes abruptly reduced to a relatively very narrow and short canal, which is interpreted as opening with the terminal portion of the uterus into a minute slit-like space at the base of a minute genital papilla (fig. 75). The latter is pierced in its long axis by a very delicate canal, interpreted as the ductus hermaphro-

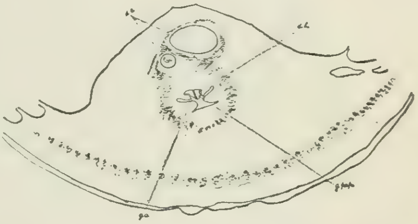


Fig. 76.

diticus. The genital papilla is interpreted as retracted into the body of the worm. Leading from it is a canal, which terminates at the surface in the genital pore. The walls of this canal are indented as seen in figure 73. When evaginated a structure is probably produced, which may in a general way resemble the corresponding structure in *Paramphist. cauliorchis.* The termination of the ductus hermaphroditicus at the vertex of the minute genital papilla is the porus hermaphroditicus, and in the evaginated condition of this terminal (copulatory) apparatus this pore would probably appear on the surface. None of the 3 specimens, the subject of this study, presented this structure in an evaginated state.

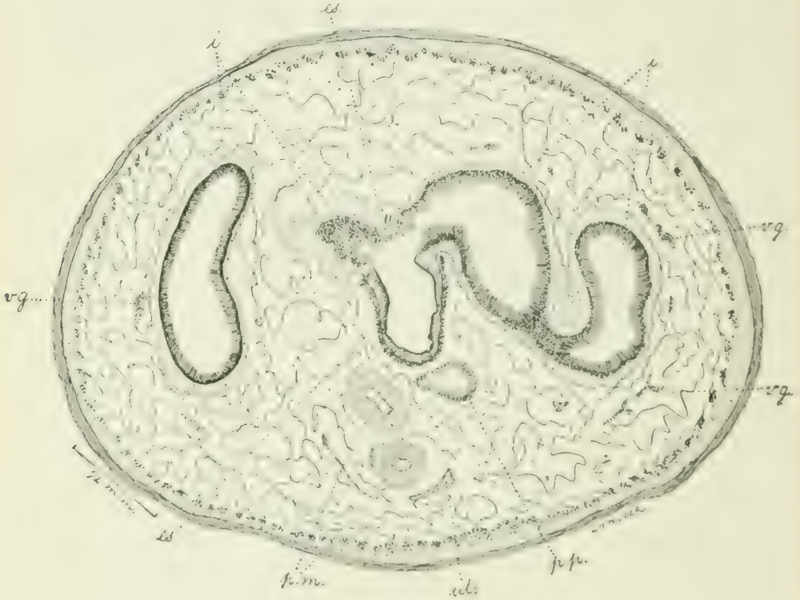


FIG. 77.

Female organs. In both of the sectioned specimens the ovary was to the right of the median line (fig. 72), considerably caudad of the right testis. Its zone overlaps the zone of caudal portion of that of the left testis to a variable extent. The oviduct arises from the caudal aspect of the ovary, passes in a more or less sinuous course to the left toward the shell gland, the right aspect of which it penetrates. The shell gland lies a little to the left of the ovary in both of the sectioned specimens. In one of these this places the shell gland in the median line, in the other slightly to the right of this line. In both of the sectioned specimens the zone of this gland slightly overlaps the caudal portion of the ovarian zone, and in one also the caudal portion of the left testicular zone. The common vitello-duct penetrates the caudal aspect of the shell gland and is joined at an angle in the subtance of the latter by the oviduct to form the ootype which is con-inued as the uterus. The latter (fig. 72) emerges from the cephalic spect of the gland, cephalad of which it describes some coils. It hen winds its way cephalad at first close to the left of the ovary, hen toward the right ventrad of the ovary. It then again turns ephalad, forming coils between the ovary and the right testis, then asses sinistro-caudad in front of the left testis, gains the left latero-entro-caudal aspect of this testis where, after forming some coils, it asses dorsad and obliquely to the right to gain a position directly entrad of the dome of the excretory vesicle. Here it turns, winding ts way cephalad, at first between the dorsum on the one hand and he left testis and ovary on the other, later for a short distance between the dorsum and the right testis. It then bends ventrad, passes to the left of the right testis beneath the transverse arch formed by the union of the vasa efferentia and over the left testis to reach the ventral aspect of the pars musculosa. The uterus now again bends and proceeds cephalad, maintaining throughout the remainder of its course the ventral relation to the pars musculosa and prostatica. Its terminal portion greatly reduced in caliber penetrates, as already described, the muscular mass inclosing the copulatory apparatus and opens immediately beneath the ductus ejaculatorius into the minute, slit-like space already described.

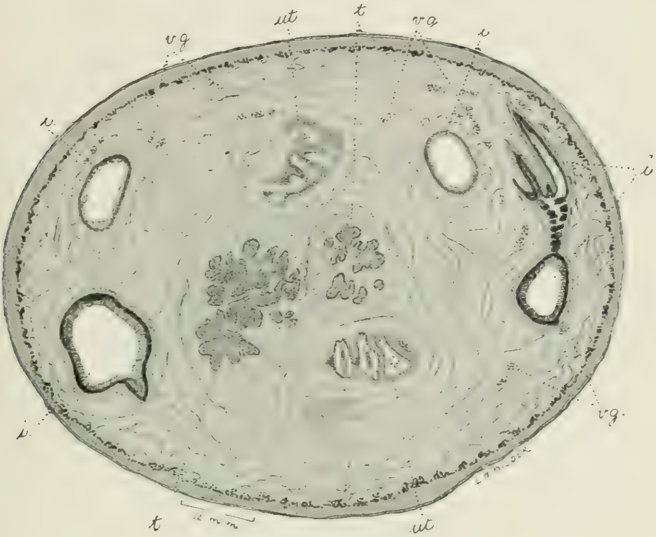


FIG. 78.

Laurer's canal (fig. 73) leaves the oviduct just as the latter is about to penetrate the shell gland. It then proceeds dorso-caudad, skirting either the right or the left aspect of the excretory vesicle in about the equatorial zone of the latter, and opens on the dorsal surface a little to the right or to the left of the median line; in one sectioned specimen, about 0.72 mm. caudad of the excretory pore.

The vitellogene glands consist of sparsely scattered follicles in the area between the intestinal ceca and the ventro-lateral, lateral, and dorso-lateral margins of the body. They extend in a vertical direction from about the level of the genital pore to a plane slightly caudad of the cecal terminations of the intestines. From each gland a duct passes more or less directly transversely inward, ventrad of the corresponding intestine, to unite with its fellow near the ventro-caudal aspect of the shell gland, between the latter and the acetabulum. From their point of union a duct, the common vitelloduct, passes to the shell gland, penetrating it on its caudal aspect as has already been described.

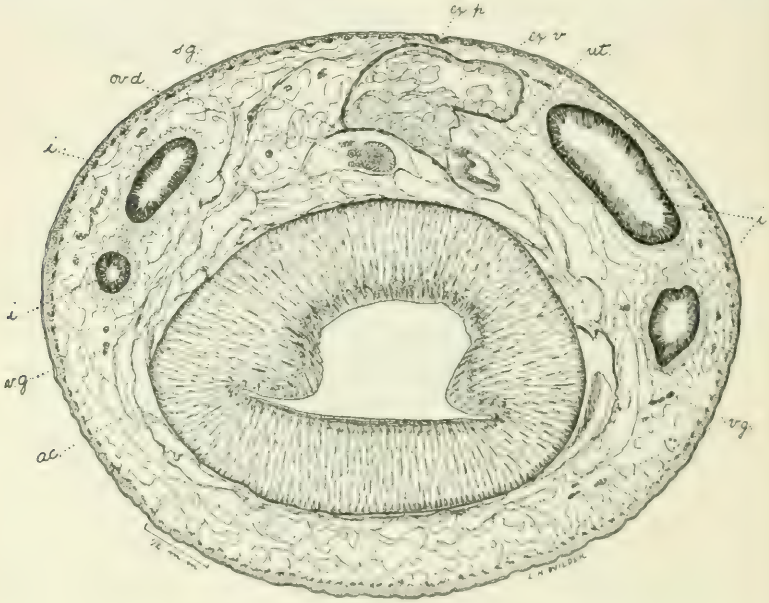


FIG. 79.

EXCRETORY SYSTEM.—The excretory system is well developed. A fairly large excretory vesicle is situated between the dorso-cephalic aspect of the acetabulum and the dorsum of the worm. A little caudad of the dome of this vesicle a thick duct leaves it and passes obliquely dorso-cephalad to open on the dorsal surface a little caudad of the superior margin of the acetabulum.

ILLUSTRATIONS.

FIG. 71.—Ventral aspect. Enlarged. Original.

FIG. 72.—Ventral projection of specimen shown in fig. 71: *ac.*, acetabulum; *g. p.*, position of genital pore; *es.*, esophagus; *i.*, intesinal ceca; *ov.*, ovary; *o. s.*, oral sucker; *s. g.*, shell gland; *t.*, testes; *t.*, uterus; *v. e.*, vasa efferentia; *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, planes of ection. Slightly diagrammatic. Enlarged. Original.

Fig. 73.—Profile projection of same: *ac.*, acetabulum; *d. e.*, ducus ejaculatorius; *es.*, esophagus; *ex. v.*, excretory vesicle; *ex. p.*, excreory pore; *i.*, intestines; *L. c.*, Laurer's canal; *ov.*, ovary; *o. s.*, oral ucker; *p. m.*, pars musculosa; *p. p.*, pars prostatica; *s. g.*, shell gland; ., right testis; *ut.*, uterus; *v. e.*, right vas efferens; *a–a*, *b–b*, *c–c*, *–d*, *e–e*, *f–f*, planes of section. Slightly diagrammatic. Enlarged. riginal.

Fig. 74.—Transverse section at *a–a* figs. 72 and 73. Shows oral ucker (*o. s.*), perisuctorial space (*p. s. sp.*) and mesenterium-like trands (*m. b.*). Enlarged. Original.

Fig. 75.—Transverse section at *b–b*, figs. 72 and 73. Shows caudal ortion of oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), ductus jaculatorius (*d. e.*), and vitellaria (*v. g.*). Enlarged. Original.

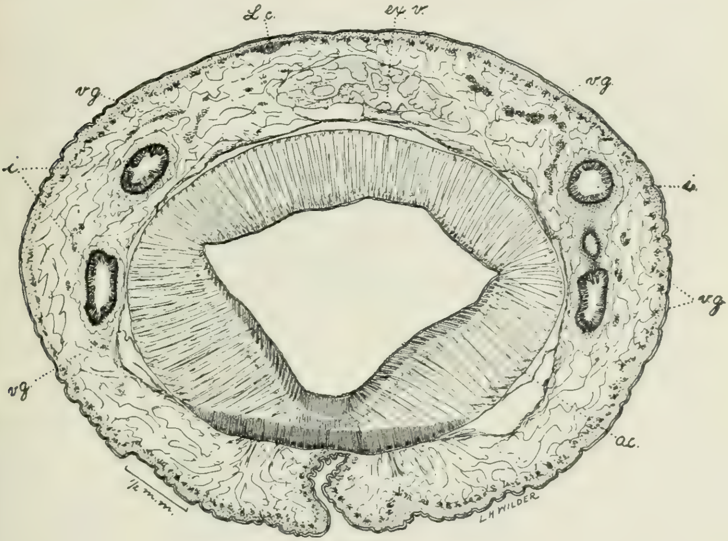


Fig. 80.

Fig. 76.—Portion of fig. 75. To show on a larger scale the copulatory apparatus: *d. e.*, ductus ejaculatorius; *d. h.*, ductus hermaphroditicus; *g. a.*, genital atrium; *g. pap.*, genital papilla. Enlarged. Original.

Fig. 77.—Transverse sections at *c–c*, figs. 72 and 73. Shows esophagus (*es.*) forking into the intestinal ceca (*i.*), the uterus (*ut.*), pars musculosa (*p. m.*), pars prostatica (*p. p.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 78.—Transverse section at *d–d*, figs. 72 and 73. Shows the testes (*t.*), uterus (*ut.*), intestinal ceca (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 79.—Transverse section at *e–e*, figs. 72 and 73. Shows excretory pore (*ex. p.*), excretory vesicle (*ex. v.*), shell gland (*s. g.*), oviduct (*ov. d.*), uterus (*ut.*), vitellaria (*v. g.*), intestinal ceca (*i.*), and acetabulum (*ac.*). Enlarged. Original.

FIG. 80.—Transverse section at *f–f*, figs. 72 and 73. Shows pore of Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), vitellaria (*v. g.*), intestinal ceca (*i.*), and acetabulum (*ac.*). Enlarged. Original.

PARAMPHISTOMUM PAPILLOSUM, new species.

[Figs. 81 to 91.]

SPECIFIC DIAGNOSIS.—*Paramphistomum* (p. 73): Body between 5.12 and 6 mm. long by 2.6 mm. in maximum breadth; gray-brown ocher tint (alcohol specimen); somewhat conical, greatest diameters (transverse and sagittal) about at border of equatorial and caudal thirds; tapers gradually to quite a blunt oral extremity; caudal extremity broadly rounded when viewed ventrally or dorsally, but beveled on its ventral aspect; lateral margins nearly straight; transverse section circular; surface coarsely grooved transversely, especially on venter; oral pole with numerous very minute conical papillæ. Genital pore ventro-median, about one-fourth the length from oral margin, and at transverse plane of caudal margin of pharynx. Acetabulum ventro-subterminal, sunken beneath surface, relatively large, 1.8 to 1.9 mm. in diameter, with small 0.36 mm. circular aperture, mouth terminal crateriform, closely beset with small digitate papillæ; oral sucker large, 0.8 mm. long, 0.9 mm. broad, 0.64 mm. in sagittal diameter, the oral two-thirds of its lumen with small papillæ; esophagus short, strongly flexed ventrad, extends beyond genital pore about to border between oral and equatorial thirds of body; intestinal ceca very long, at first transverse, then turned caudad and extend in very wavy course, each about midway between median line and corresponding lateral margin, more dorsal than ventral, nearly or quite to aperture of acetabulum; slings distinctly dorso-ventral and somewhat parallel to body wall. Excretory pore dorso-median, slightly caudad of cephalic margin of acetabulum and cephalad of Laurer's canal; excretory vesicle well developed, broad; excretory canal arises about from equator of vesicle, short, runs directly dorsad to its pore.

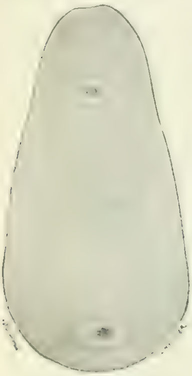


FIG. 81.

Male organs.—Testes deeply branched, cauliflower like, in axis of equatorial region of body, close together one caudo-dorsad of the other; each vas efferens arises from lateral aspect, passes cephalo-dorsad, then mediad, uniting with its fellow somewhat cephalo-dorsad of anterior testis to form the much-coiled vesicula seminalis; pars musculosa thick walled, coiled, ventral of vesicula; pars prostatica relatively straight, passes ventro-cephalad, ventral of esophagus, is continued in a short ductus ejaculatorius which opens into dilated ductus hermaphroditicus; the latter pierces the axial region of genital papilla, which is separated from a papillated cylindrical antechamber by a nonpapillated ridge or ring; the papillated chamber opens externally through the apparent genital pore. Cirrus pouch absent.

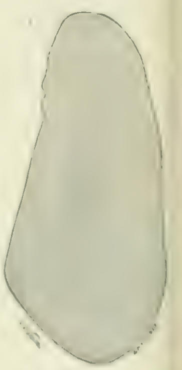
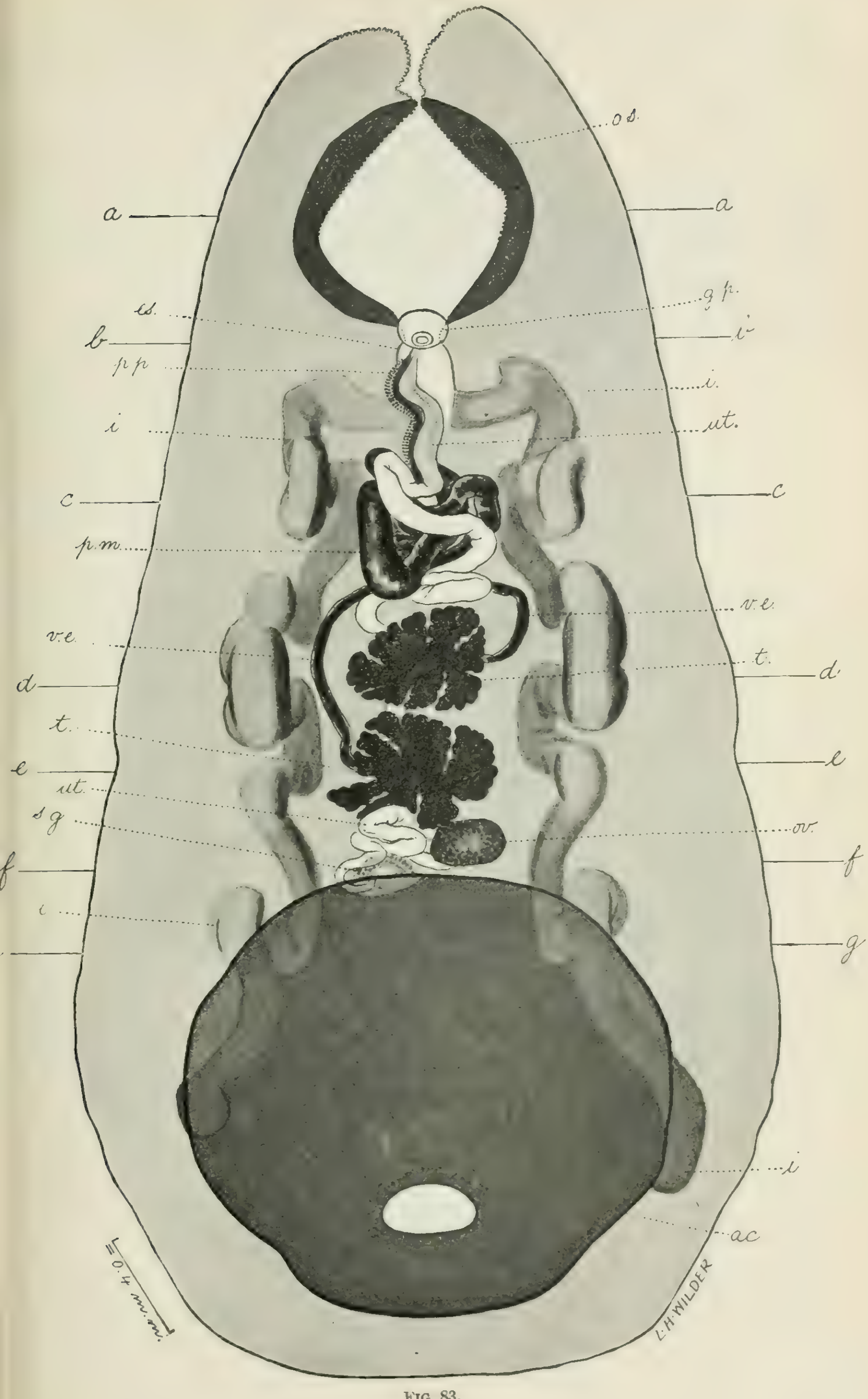


FIG. 82.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L.H. WILDER

FIG. 83.

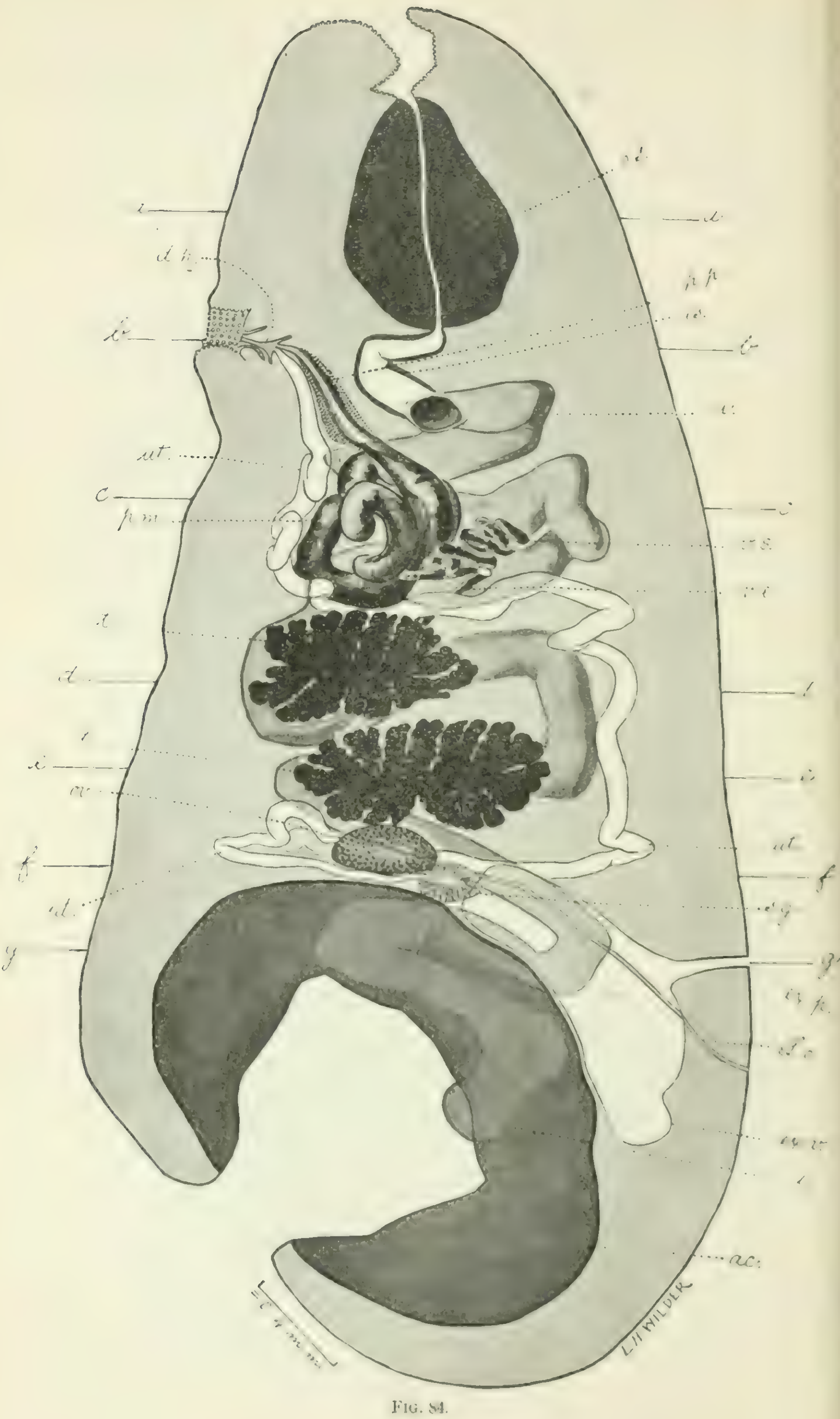


FIG. 84.

Female organs.—Ovary slightly sinistral, slightly ventral, immediately cephalad of acetabulum; shell gland smaller than and dorso-dextro-caudal of ovary; also slightly dextral of median line; vitellaria with sparsely scattered small follicles, lateral of ceca, extending from base of oral sucker to caudal end of ceca; uterus passes from shell gland ventrad caudally of ovary, then coils ventrally of ovary, then passes dorsad between shell gland and testis on right of ovary, dorsally around testes, ventrad beneath vasa efferentia, cephalad ventrally of vas deferens, opening ventrally of ductus ejaculatorius into ductus hermaphroditicus. Laurer's canal runs dorso-caudad at right of excretory vesicle to a point 0.4 mm. caudad of excretory pore, and slightly dextral of median line.

Eggs.—Not observed.

TYPE.—U.S.B.A.I. 15025, sectioned.

HOST.—The Zebu (*Bos indicus*) at Sanawaar, Punjab, India.

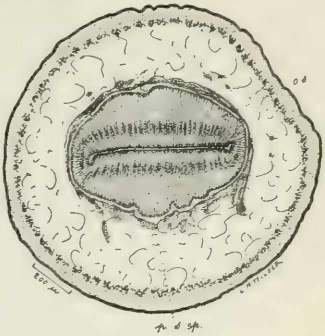


FIG. 85.

SOURCE OF MATERIAL.—This single specimen was found in a bottle with other forms bearing the B. A. I. No. 1723; it was renumbered 15025. The label in this bottle reads as follows:

Name *Amphistomum crumeniferum.* Host *Bos indicus.* Locality Sanawaar, Punjab, India. Collected by Doctor Giles. Date III. 1893. Determined by Dr. Giles. Date III. 1893. Presented by Dr. Giles. Date III. 1893.

EXTERNAL CHARACTERS.

SIZE.—Measured from sections the worm was 5.12 mm. long and 2.6 mm. in greatest width; in alcohol it was 6 mm. long.

COLOR.—The worm was of a gray-brown ocher tint.

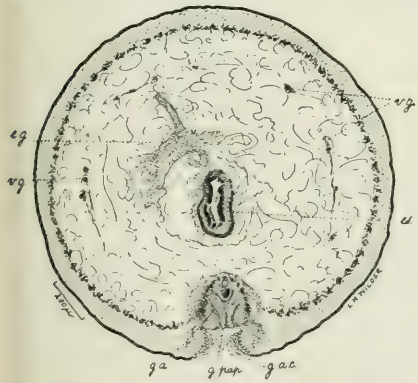


FIG. 86.

FORM.—The form of this worm is well shown in figs. 81 and 82. The worm has a somewhat conical shape, having its greatest transverse and dorso-ventral diameters in the region of junction of the middle with the caudal third whence it tapers gradually to quite a blunt oral extremity. The aboral pole is broad and rounded from side to side, but beveled on its ventral aspect where it presents the aperture of the acetabulum. In transverse section the outline is circular.

SURFACE.—The surface cuticle is coarsely grooved transversely, particularly on the venter. At the oral pole there appear to be numerous very minute conical papillæ.

Genital pore.—In the median longitudinal line of the venter about one-fourth the length from the oral extremity is a small aperture, the genital pore, measuring about 0.20 mm. in diameter.

Acetabulum.—The acetabulum is in the caudal portion of the body, distinctly sunken beneath the body surface in the specimen examined. It is relatively large, measuring about 1.8 to 1.9 mm. in diameter, but is provided with a rather small circular aperture which measures 0.36 mm. in diameter; the portion of the rim of the aperture formed by the body of the worm (as distinguished from the portion formed by the acetabulum) is slightly puckered (fig. 82).

INTERNAL ANATOMY.

Digestive tract.—The oral pole of the worm presents an irregularly crateriform depression, closely beset by numerous, small digitate papillæ. This depression leads by a minute irregularly circular aperture into a large muscular oral sucker. The latter is about 0.86 mm. long, about 0.90 mm. in greatest width and 0.64 mm. in greatest ventro-dorsal diameter; it lies in a cavity (fig. 85), suggestive of a rudimentary body cavity, and is bound to the body parenchyma at its oral and aboral poles and by distinct mesenterium-like bands on both its dorsal and its ventral median aspects. In median sagittal section it is somewhat pyriform in outline; in frontal section it is almost circular, while in transverse section (fig. 85) it is elliptical in outline. The muscular wall is thickest in the ventro-dorsal and thinnest in the transverse axis. The lumen is a transversely broad, dorso-ventrally narrow slit-like space and is lined by a thin cuticle-like layer seemingly (no sagittal sections available for confirmation) in anatomical continuity with the surface cuticle. In about the upper (cephalic) two-thirds of the lumen the lining is beset by very small slender conical papillæ; these give to the cuticle a serrate appearance (transverse sections), with the papillæ of the dorsal wall fitting into the interspaces between the papillæ of the ventral wall.

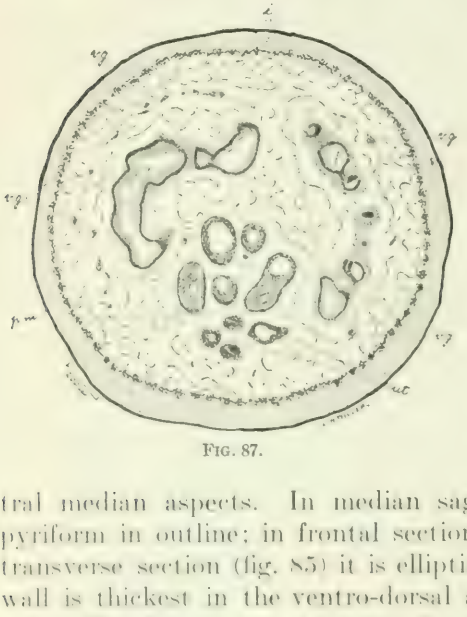


Fig. 87.

At the two extremities of the oral sucker the lumen becomes contracted so as to form at each end a small circular aperture; at the caudal extremity this aperture gives entrance into the esophagus; very slightly caudally of this point may be distinguished a well developed ganglion-complex (fig. 86), lying dorsally of the esophagus and sending off strong nerve strands in various directions. The esophagus passes from the base of the sucker and describing a U-shaped course with the base of the U ventrad, divides into two intestines at about the level of junction of the cephalic with the middle third of the body. The intestinal tubes pass one to the right and the other to the left in a transverse plane latero-dorsad to a point about midway between the longitudinal axis and the body wall; they then pursue a wavy course caudad (figs. 83, 84), to terminate by cecal extremities latero-dorsad of the acetabulum about in the transverse plane of the upper (cephalic) margin of the aperture of the acetabulum. In their wavy course caudad the ceca continue in a general way about midway between the longitudinal body axis and the body wall but approaching closer to the dorsal than to the ventral median longitudinal line; furthermore, the slings or coils have a distinctly dorso-ventral direction (figs. 84, 87, 89, 90) and run somewhat parallel to the body wall. The lumen of the esophagus is lined by a thick cuticular layer, which ceases abruptly at the point of origin of the intestines. The latter are lined by an epithelial cell layer.

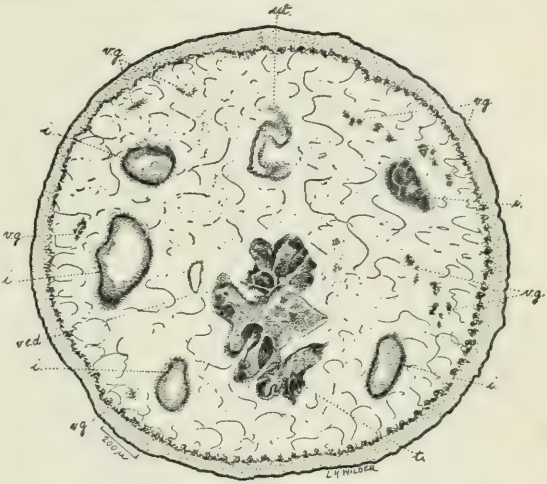


FIG. 88.

GENITAL SYSTEM.—*Male organs.*—The testes, one caudad of the other, are in the axial portion of the equatorial region of the body, though in general slightly nearer the venter than the dorsum (figs. 84, 88, 89); of the two, the superior (cephalic) testis is nearer the venter, and, being somewhat smaller, is also farther from the dorsum than is the inferior (or caudal) testis. The opposing aspects of the testes are closely approximated. Both testes are much branched, suggesting a cauliflower in appearance (figs. 88, 89). (The projections do not show this well.) From the right lateral aspect of the caudal, and from the left lateral aspect of the cephalic testis there spring the corresponding vasa efferentia; these pass cephalo-dorsad, then mediad, describing an arch as they unite a little above the superior testis to form the vas deferens (fig. 84). Beneath this arch, and between it and the superior testis, the uterus passes ventrad. The vas deferens presents at first a much coiled thin walled portion, (vesicula) the lumen of which in the single specimen studied was not noticeably dilated. This is succeeded by a coiled quite thick walled (45μ) portion, pars musculosa, measuring about 150μ in diameter, which is situated ventro-cephalad of the coiled thin-walled portion. The caliber of this muscular portion appears somewhat greater than that of the vesicula. The muscular portion is succeeded by a relatively straight pars prostatica of moderate length, the walls of which, though thick and muscular at first, rapidly become thinned and are inclosed in a mass of cells. This portion passes ventro-cephalad, being in relation to the ventral aspect of the esophagus, and in still closer relation to the dorsal aspect of the terminal portion of the uterus; it is finally succeeded by a short duct (by homology, the ductus ejaculatorius) not inclosed in prostatic cells and opens by a minute pore immediately above the metraterm into a small slit-like atrium at the base of the genital papilla. This atrium may be regarded as the dilated dorsal portion of the ductus hermaphroditicus which pierces the axial region of the genital papilla. The genital papilla is embraced by a ring, one aspect of which is applied to it and separated from it only by a narrow slit; the other aspect forms the dorsal wall of a relatively large cylindrical atrium (figs. 84, 86); this latter is beset by small papillæ (hence the specific name *papillosum*), which do not occur, however, on either surface of the ring embracing the genital papilla. The genital pore gives entrance and exit to this chamber, and, as will be seen from figure 84, it is at or slightly caudad of the level of the base of the oral sucker.

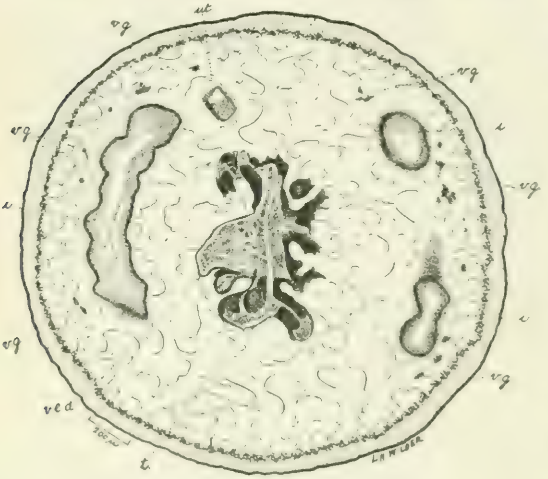


Fig. 89.

Female organs. The ovary is in the axial region of the body, though a little to the left of the median sagittal plane, and a little nearer to the venter than to the dorsum and just above (cephalad of) the acetabulum. From its dorso-median aspect there arises the oviduct which passes to the right and dorso-caudad toward the shell gland. The shell gland, somewhat smaller than the ovary, lies a little to the right, and a little dorso-caudad of the ovary (figs. 83, 84, 90) and also slightly to the right of the median sagittal plane of the body of the worm. It is penetrated on its dorso-median aspect by the oviduct and on its dorso-caudal aspect by the vitello-duct. These unite in the substance of the shell gland to form the ootype, which is continued as the uterus, the latter emerging from the ventral aspect of the gland (fig. 90). Laurer's canal leaves the oviduct just before the latter penetrates the shell gland; it then passes to the right and dorso-caudad, skirting the upper portion of the right lateral aspect of the excretory vesicle (figs. 84, 91), and opens on the dorsum about 0.40 mm. caudad of the excretory pore, slightly to the right of the median line.

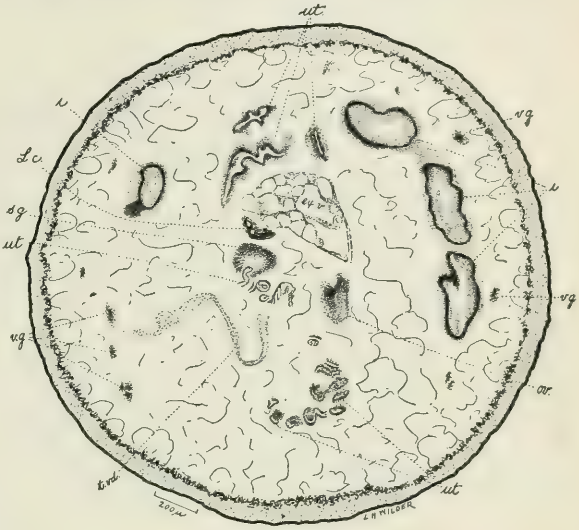


FIG. 90.

The vitellogene glands, composed of sparsely scattered insignificant follicles, lie in the lateral regions of the body between the intestinal ceca and the body walls. They extend longitudinally from about the level of the base of the oral sucker to the level of the termination of the intestinal ceca. From each gland there arises a duct, which passes transversely inward ventrally of the intestines; the two unite at the level of the superior margin of the acetabulum and close to the ventro-median aspect of the shell gland. From their point of union a duct is given off which skirts the caudal aspect of the shell gland, penetrating the latter at the caudo-dorsal aspect.

The uterus, as already described, emerges from the ventral aspect of the shell gland (fig. 90), passes ventrad, then for a short distance cephalad forming some coils ventrally of both ovary and shell gland; it then passes dorsad to the right of the ovary and between the shell gland and caudal testis. Having reached the space between the caudal testis and dorsum it passes cephalad behind (dorsally of) both testes, then ventrad above (cephalad of) the superior testis and beneath the arch of union of the vasa efferentia, to gain the ventral aspect of the coil formed by the pars musculosa. It next passes in a general way cephalad and reaches the ventral aspect of the pars prostatica; this relation it maintains in the remainder of its course ventro-cephalad to terminate by a minute pore immediately beneath the pore of the ductus ejaculatorius in the manner already described. In its course the uterus is moderately coiled. Neither eggs nor spermatozoa were observed in its lumen.

EXCRETORY SYSTEM.—The excretory vesicle (figs. 84, 90, 91) lies dorsally of the acetabulum between the terminal portions of the intestinal ceca. Its transverse diameter is greater than its ventro-dorsal diameter; it discharges by a thick walled duct which arises from about the middle of its dorsal aspect; this duct passes almost directly dorsad to open, in the median line, somewhat (about 0.28 mm.) below the level of the upper margin of the acetabulum, and about 0.4 mm. cephalad of the pore of Laurer's canal.

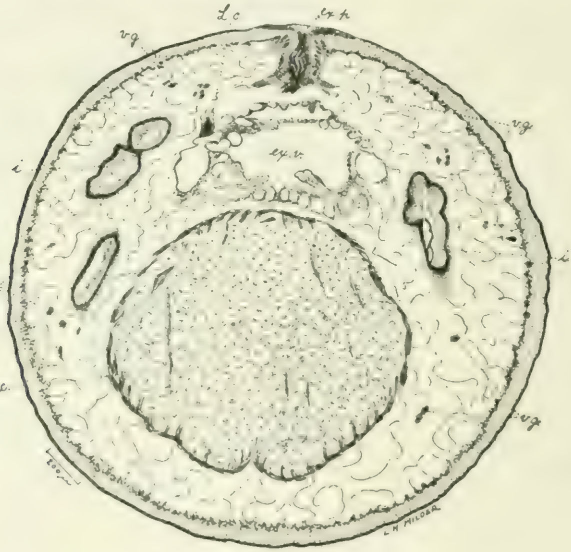


FIG. 91.

ILLUSTRATIONS.

FIG. 81.—Ventral aspect. Enlarged. Original.

FIG. 82.—Profile of same. Enlarged. Original.

FIG. 83.—Ventral projection of specimen shown in figs. 81 and 82: *ac.*, acetabulum; *g. p.*, genital pore; *es.*, esophagus; *i.*, intestinal ceca; *ov.*, ovary; *o. s.*, oral sucker; *p. m.*, pars musculosa; *p. p.*, pars prostatica; *s. g.*, shell gland; *t.*, testes; *ut.*, uterus; *v. e.*, vasa efferentia. *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 84.—Profile projection of specimen shown in figs. 81 and 82: *ac.*, acetabulum; *d. h.*, ductus hermaphroditicus; *es.*, esophagus; *ex. p.*, excretory pore; *ex. v.*, excretory vesicle; *i.*, intestine; *L. c.*, Laurer's canal; *ov.*, ovary; *o. s.*, oral sucker; *p. m.*, pars musculosa; *p. p.*, pars prostatica; *s. g.*, shell gland; *t.*, testes; *ut.*, uterus; *v. e.*, vas efferens; *v. s.*, vesicula seminalis; *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 85.—Transverse section at *a–a*, figs. 83 and 84. Shows oral sucker (*o. s.*), and perisuctorial space (*p. s. sp.*). Enlarged. Original.

Fig. 86.—Transverse section through *b–b* figs. 83 and 84. Shows papillated genital ventral chamber (*g. a. c.*), genital dorsal chamber (*g. a.*), genital papilla (*g. pap.*), esophagus (*es.*), esophageal ganglion (*e. g.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 87.—Transverse section at *c–c* figs. 83 and 84. Shows uterus (*ut.*), pars musculosa (*p. m.*), intestinal ceca (*i*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 88.—Transverse section at *d–d* figs. 83 and 84. Shows superior testis (*t.*), intestines (*i.*), uterus (*ut.*), right vas efferens (*v. e. d.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 89.—Transverse section at *e–e* figs. 83 and 84. Shows caudal testis (*t.*), origin of right vas efferens (*v. e. d.*), uterus (*ut.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 90.—Transverse section at *f–f* figs. 83 and 84. Shows uterus (*ut.*), right transverse vitello-duct (*t. v. d.*), caudal portion of ovary (*ov.*), shell gland (*s. g.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 91.—Transverse section at *g–g* figs. 83 and 84. Shows acetabulum (*ac.*), intestines (*i.*), excretory vesicle (*ex. v.*), Laurer's canal (*L. c.*), excretory pore (*ex. p.*), and vitellaria (*v. g.*). Enlarged. Original.

PARAMPHISTOMUM INDICUM, new species.

[Figs. 92 to 102.]

Specific diagnosis.—*Paramphistomum* (p. 73): Body 5.25 to 9.5 mm. long by 2 to 3 mm. in maximum breadth; gray-brown ochre (alcohol specimens); somewhat conical, greatest transverse diameter about at border of equatorial and caudal thirds; tapers moderately to blunt, almost truncated oral extremity; caudal extremity rounded on ventral view, but with tendency to beveled ventro-lateral aspect on lateral view; lateral margins nearly straight to slightly curved; body bent, with venter rather strongly concave (longitudinally) and dorsum convex; transverse section circular anteriorly, but with greater transverse than dorso-ventral diameter from near genital pore for some distance caudad, then the dorso-ventral diameter becomes the greater. Surface coarsely wrinkled transversely; cephalic half provided with irregular, transverse rows of papillæ which are more numerous, acutely conical in circumoral region, but more scattered, shorter, broader, and blunt elsewhere. Genital pore ventromedian about at border between cephalic and equatorial thirds of body, caudad of esophagus, but nearer to this than to anterior testis. Acetabulum terminal, but aperture appears ventro-subterminal because of curvature of body, sunken beneath surface, rather large, 1.6 mm. in longitudinal diameter, very slightly less in transverse diameter, with rather circular to somewhat irregular, 0.53 mm. to 0.65 mm. aperture which appears ventrad and slightly caudad. Mouth terminal, hour-glass form, provided with papillæ; oral sucker large, oval, 0.96 mm. long, 0.84 mm. broad, 0.58 mm. in greatest dorso-ventral diameter, the oral two-thirds of its lumen with small blunt papillæ; esophagus about half as long as sucker, at first directed caudad, then suddenly dorsad, does not extend to genital pore; intestinal ceca long, pursue dorso-ventrally wavy course about to anterior margin of aperture of acetabulum. Excretory pore dorso-median, cephalad of acetabulum, about on caudal plane of caudal testis, and about 1 mm. cephalad of pore of Laurer's canal; excretory duct short, runs from pore to dorso-cephalic aspect of very large excretory vesicle which lies dorsally of cephalic portion of acetabulum.

Fig. 92.

Male organs: Testes deeply notched (lobulated), in transverse section cauliflower like, in axis of body, one caudad of the other, slightly caudad of equatorial plane, very slightly nearer to venter than to dorsum, one very slightly nearer each lateral margin than is the other; each vas efferens arises from dorsal aspect of testis, passes cephalo-mediad, unites with its fellow about 0.5 mm. cephalad of superior testis to form vas deferens; vesicula seminalis and pars musculosa both much coiled and extend in ventrocephalic direction; pars prostatica short, nearly straight, directed ventrad; pars ejaculatorius very short, unites with metraterm dorsally of pore to form ductus hermaphroditicus, which pierces a small papilla to discharge into a narrow nonpapillated atrium, which in turn discharges at the genital pore; cirrus pouch absent.

Female organs: Ovary right or left of median line, just cephalad of acetabulum; shell gland smaller, dorso-median and slightly caudad of ovary; vitellaria with sparsely scattered, very small follicles, lateral to dorso-lateral of ceca, from base of oral sucker to cephalic margin of acetabulum (not quite to end of ceca); uterus passes in wavy course ventrally of shell gland, dorsad caudally of posterior testis, cephalad of both testes, ventrad under arch of vasa efferentia, cephalad ventrally of vas deferens, to ductus hermaphroditicus; Laurer's canal arises from oviduct about midway between ovary and shell gland, runs dorso-caudad at right of excretory vesicle to pore situated on dorsum, 0.8 to 1.0 mm. caudad of excretory pore, and either median or very slightly to the right of median line.

Eggs: Not observed.

Type.—U.S.B.A.I. 1723; cotype U.S.N.M. 5775.

Host.—The Zebu (*Bos indicus*) in Punjab, India.

Source of material.—These worms were found with some other forms in 2 bottles, bearing the numbers "B.A.I. 1723" and "U.S.N.M. 5775," respectively. The other material in question has been separated out and renumbered.

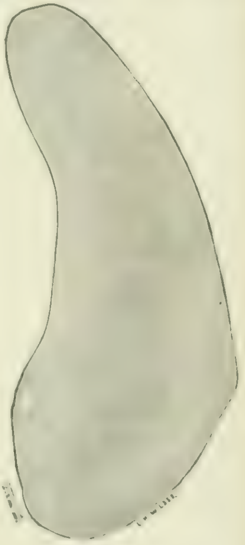
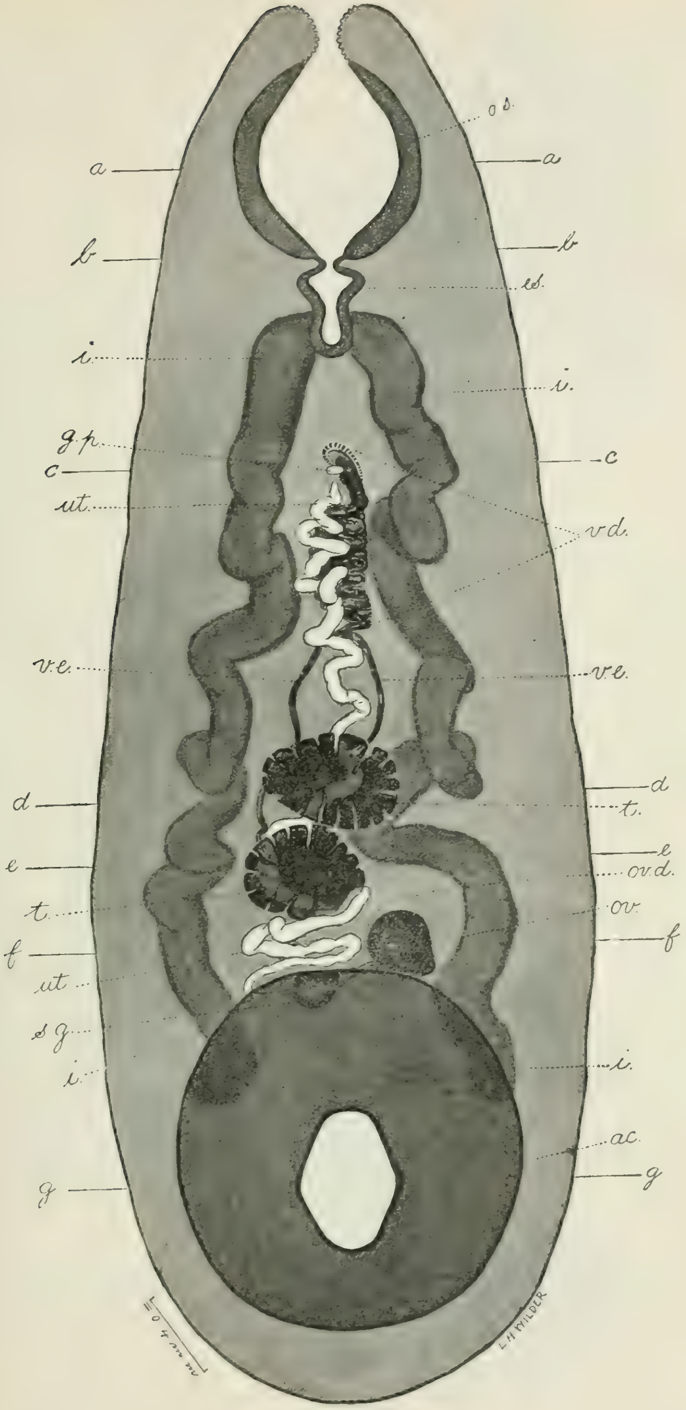


Fig. 93.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L.H.WILDER

FIG. 94.

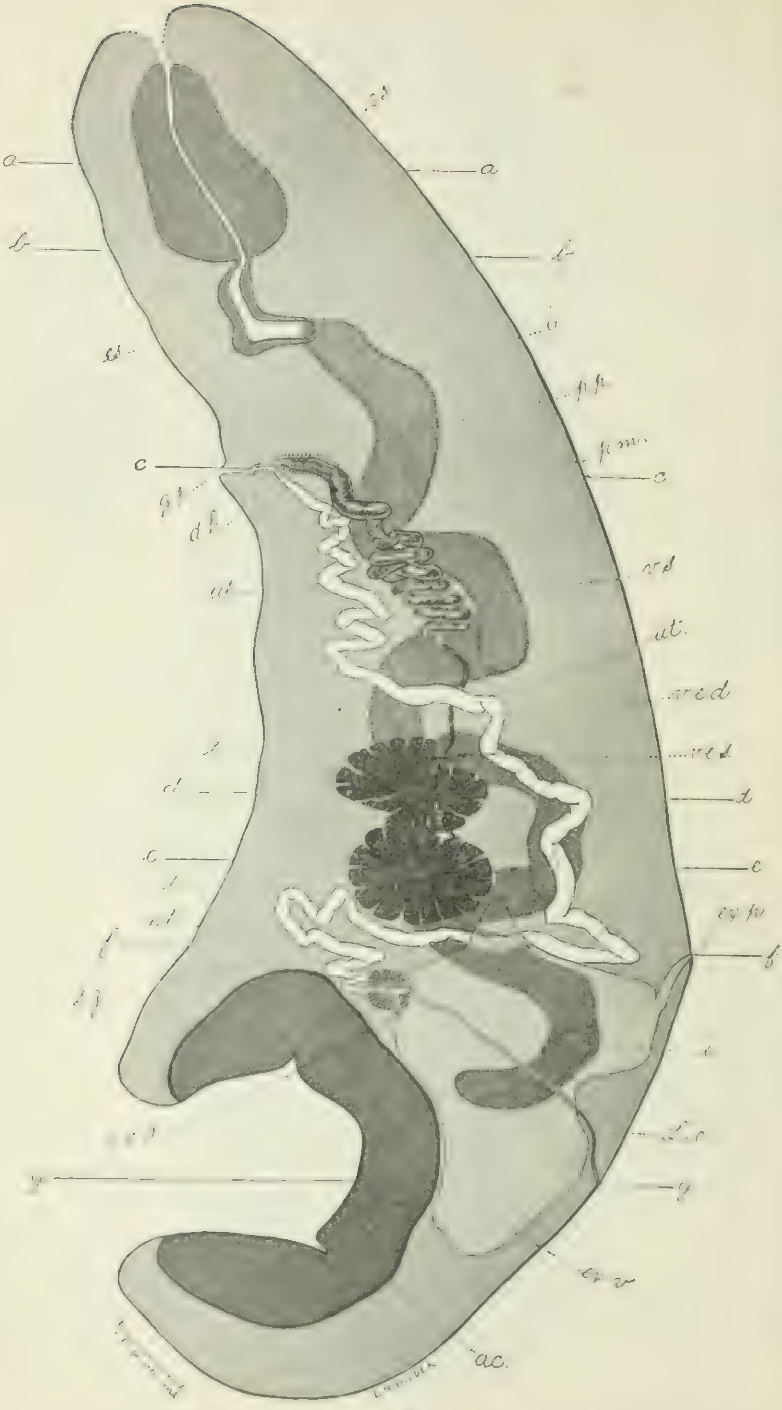

FIG. 95.

EXTERNAL CHARACTERS.

Size.—The alcohol preserved specimens—14 in all—measured from 5.25 to 9.5 mm. in length. On account of the damaged condition of 6 of them the width could be measured in 8 only, and in these the maximum breadth varied from 2 to 3 mm.

Color.—The worms are of a gray-brown ocher.

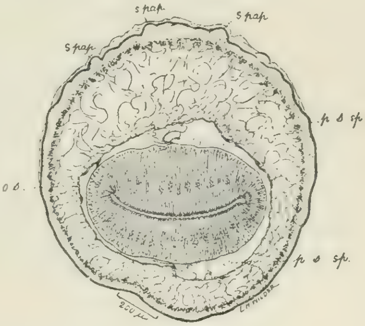

Fig. 96.

Form.—The specimens are somewhat conical in form (figs. 92, 93) and the ends are bent more or less ventrally, so that the longitudinal axis is a curve with a concave venter and a strongly convex dorsum. The greatest transverse diameter is near the junction of the equatorial with the caudal third of the body; the worm tapers moderately to a blunt, and in some specimens, slightly bulbous oral extremity. On transverse section the cephalic portion is circular, but beginning slightly cephalad of the genital pore the outline undergoes a slight change, the transverse diameter becoming greater than the dorso-ventral diameter; near the caudal end the outline again changes, the dorso-ventral diameter being the greater.

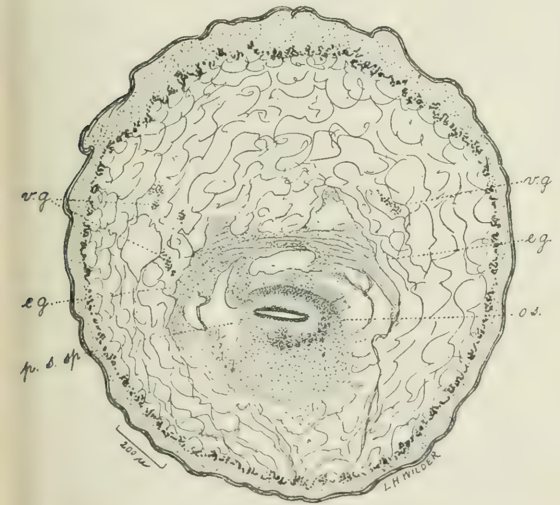

Fig. 97.

Surface.—The surface of the worms, except for some transverse wrinkling, is smooth in the caudal half of the animal; in the cephalic half, however, the cuticle is provided with numerous papillæ, which are acutely conical in the circumoral region, but relatively shorter, broader, and more blunt over the remaining portion of the surface covered by them; at least some of these appear to be of sensory nature. The papillæ around the oral aperture are fairly numerous and closely aggregated; the others are relatively less numerous, more scattered, and arranged in irregular transverse rows.

Genital pore.—In the median longitudinal line of the ventral surface, at about the junction of the cephalic with the middle third of the body, is the genital pore (figs. 94, 95, 98). This gives entrance into a canal which leads into a small nonpapillated atrium, the dorsal wall of which is formed by a short papilla in the center of which is the porus hermaphroditicus. Sections show the genital atrium to be inclosed in considerable muscular mass (figs. 95, 98).

Acetabulum.—This is terminal anatomically, but appears ventro-subterminal because of the curvature of the body. In one of the sectioned specimens it measured about 1.6 mm. in greatest (longitudinal) diameter. Its more or less circular aperture is directed ventrad and slightly caudad, appearing ventro-subterminal, and in 7 specimens varies from 0.53 to 0.65 mm. in diameter.

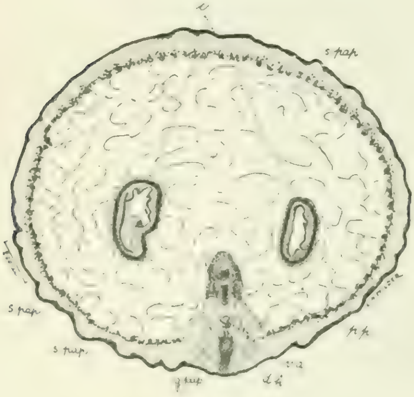

Fig. 98

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The mouth, which pierces the cephalic blunt extremity, leads into a large muscular oral sucker of oval form and rather similar to that of *P. papillosum*. The cephalic two-thirds of the lumen of this sucker is beset with small but distinct bluntly pointed conical papillæ (fig. 96); in the esophageal third there is a slight indication of much more minute papillæ. The sucker lies in a cavity suggestive of a rudimentary body cavity, held in place by its attachments at its poles and by mesenterium-like bands extending from the dorsal and ventral aspects through the cavity to the body parenchyma. The sucker leads into the esophagus, and dorsally of the point of union there is a distinct nervous band giving off branches in various directions (fig. 97). At first the esophagus passes almost directly caudad, then bending at almost right angles it is directed for the remainder of its short course dorsad to fork into two intestinal ceca. The length of the esophagus is equal to about one-half of that of the sucker. The intestinal ceca pursue a dorso-ventral wavy course (approximately parallel to the body wall) caudad to terminate dorsally of the acetabulum and in a transverse plane just above (cephalad) of the acetabular aperture (figs. 94, 95).

GENITAL SYSTEM.—*Male organs.*—The testes are in the axial region of the cephalic portion of the caudal half of the body, between the equatorial plane and the plane of the cephalic margin of the acetabulum (figs. 94, 95), somewhat nearer to the venter than to the dorsum. The testes are deeply indented or lobulated, so that in transverse section they appear as if branched in cauliflower-like fashion (figs. 99, 100). They are situated almost directly caudad one of the other, their opposing surfaces being either in the same or in slightly separated transverse planes. From the dorsal aspect of each testis arises a vas efferens; in one (figs. 94, 95) of the two sectioned specimens the right vas belonged to the inferior, and in the other to the superior testis. The vasa efferentia pass cephalad, and about 0.5 mm. above the superior testis unite in the axial region of the body to form the much coiled vas deferens. The first portion of the latter is thin walled (vesicula), and its general course is ventro-cephalad. The second portion, also much coiled, is provided with thick muscular walls. The third portion of the vas deferens is short, fairly straight, directed almost horizontally ventrad, and its walls are inclosed in a mass of cells; this, the pars prostatica, becomes suddenly greatly reduced both in the caliber of its lumen and the thickness of its walls, and now devoid of prostatic cells this terminal (fourth) portion represents a very short ductus ejaculatorius, which unites with the metraterm to form a short ductus hermaphroditicus (fig. 95).

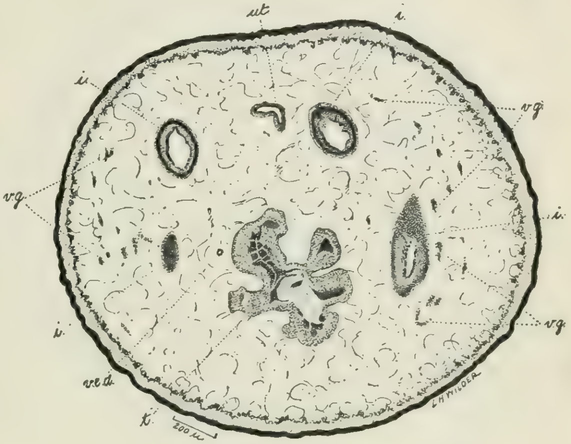

FIG. 99.

Female organs.—The ovary lies in the superior (cephalic) part of the caudal third of the axial region of the body (fig. 101), a little either to the right or to the left of the median sagittal plane. Its cephalic aspect is in the same transverse plane as, or in the plane immediately caudad of, the plane of the caudal aspect of the caudal testis. The shell gland lies on the dorso-median aspect of the ovary, slightly caudad of and either close to or somewhat removed from the latter. The oviduct springs from the ovary from the aspect nearest the shell gland in one specimen, but very slightly more dorsally in another; it passes toward the shell gland which it penetrates. In the substance of the shell gland the oviduct unites with the vitelline duct to form the ootype. Laurer's canal leaves the oviduct at a point about midway between the ovary and the shell gland, or slightly nearer the ovary, and passes dorso-caudad, skirts the right side of the excretory vesicle (both in the specimen with dextral and in the one with sinistral ovary), and reaches the dorsal body surface at a point about on a level with the middle of the acetabular aperture and between 0.80 mm. and 1 mm. caudad of the excretory pore. In the specimen with the dextral ovary the pore of Laurer's canal is median, while in the specimen with sinistral ovary it is very slightly to the right of the median line. The vitellogene glands, consisting of sparsely scattered insignificant follicles, lie in the lateral and dorso-lateral regions of the body, between the intestinal ceca and the body surface, and extend longitudinally between the plane of the base of the sucker and that of the upper margin of the acetabulum. The transverse vitello-ducts unite dorsad of the acetabulum and immediately caudad of the shell gland. From their point of union, which is not dilated into a reservoir, a duct springs which penetrates the caudal aspect of the shell gland.

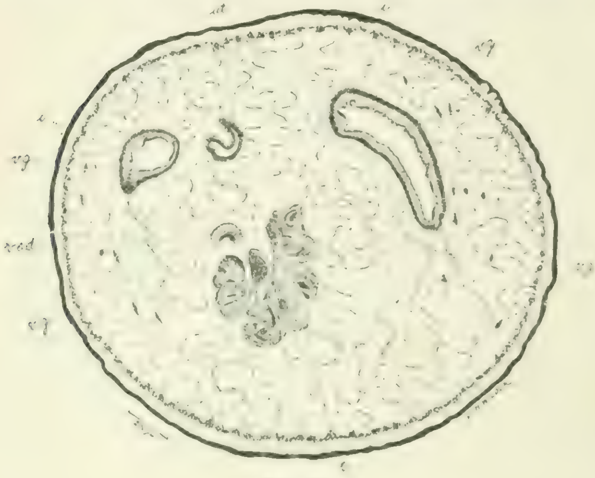

FIG. 100.

The uterus, as a continuation of the ootype, emerges from that ventro-lateral aspect of the shell gland which is away from the ovary, and then forms coils ventrad of the shell gland and ovary as it winds its way for a short distance cephalad before it turns dorsad beneath the caudal testis. In its course dorsad it passes to the right of the upper portion of the excretory vesicle. On reaching the region dorso-caudally of the caudal testis it resumes its course cephalad between the dorsum and the testes; just above the plane of the cephalic aspect of the superior testis it bends ventro-cephalad, passing beneath the arch formed by the union of the vasa efferentia, and gains the ventral aspect of the vas deferens; this relation it maintains in the remainder of its course, its terminal portion or metraterm uniting with the ductus ejaculatorius to form the ductus hermaphroditicus. Eggs were not observed in the uterus of either of the specimens studied.

Excretory system.—The excretory vesicle is large and lies dorsally of the acetabulum, its fundus extending caudad to a plane somewhat above the caudal margin of the acetabulum. The vesicle opens by a short duct which leaves it from the dorso-cephalic aspect and opens on the dorsum a little above the plane of the upper margin of the acetabulum and as already stated almost 1.00 mm. cephalad of the pore of Laurer's canal.

RELATION TO KNOWN SPECIES.

This species resembles most closely *Paramphist. gracile* Fischœder. It differs from the latter in possessing an esophagus that is only about one-half as long as the sucker; in the position of the testes which in this species are placed relatively farther caudad than in *P. gracile;* in the position of the ovary which in this form is separated little, if at all, in a longitudinal direction from the caudal testis; and in the position of the pore of Laurer's canal which in this form is very much farther caudad, opening about opposite the middle of the acetabular aperture whereas in *P. gracile* it opens in the plane of the superior aspect of the ovary some distance above the upper margin of the acetabulum.

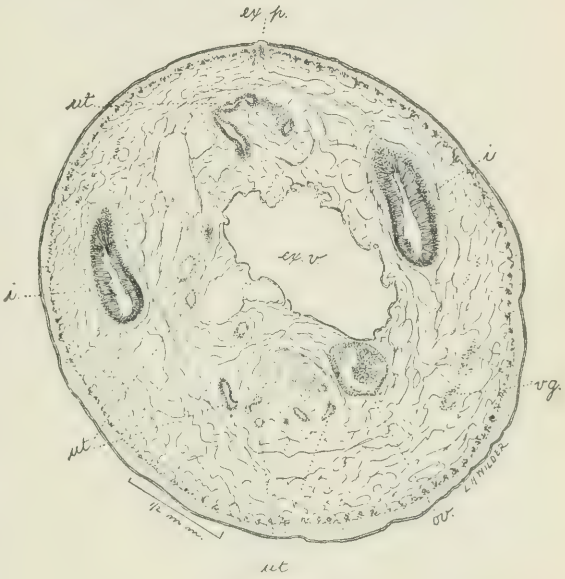

Fig. 101.

ILLUSTRATIONS.

Fig. 92.—Ventral aspect. Enlarged. Original.

Fig. 93.—Profile view of same. Enlarged. Original.

Fig. 94.—Ventral projection of specimen shown in figs. 92 and 93: *ac.*, acetabulum; *g. p.*, genital pore; *es.*, esophagus; *i.*, intestines; *ov.*, ovary; *ov. d.*, oviduct; *o. s.*, oral sucker; *s. g.*, shell gland; *t.*, testes; *v. d.*, vas deferens; *v. e.*, vasa efferentia; *ut.*, uterus. *a–a*, *b–b*, *c–c*,

d–d, *e–e*, *f–f*, *g–g*, planes of section. Slightly diagrammatic. Enlarged Original.

FIG. 95.—Profile projection of specimen shown in figs. 92 and 93: *ac.*, acetabulum; *g. p.*, genital pore; *c. vd.*, common vitello-duct; *es.*, esophagus; *d. h.*, ductus hermaphroditicus; *ex. p.*, excretory pore; *ex. v.*, excretory vesicle; *i.*, right intestinal cecum; *L. c.*, Laurer's canal; *o. s.*, oral sucker; *p. m.*, pars musculosa; *p. p.*, pars prostatica; *s. g.*, shell gland; *t.*, testes; *v. e. d.*, right vas efferens; *v. e. s.*, left vas efferens; *v. s.*, vesicula seminalis; *ut.*, uterus. *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, planes of section. Slightly diagrammatic. Enlarged. Original.

FIG. 96.—Transverse section at *a–a* figs. 94 and 95. Shows surface papillæ (*s. pap.*), oral sucker (*o. s.*), and perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 97.—Transverse section at *b–b* figs. 94 and 95. Shows base of oral sucker (*o. s*), perisuctorial space (*p. s. sp.*), esophageal ganglion (*e. g.*), and vitellogene glands (*v. g.*). Enlarged. Original.

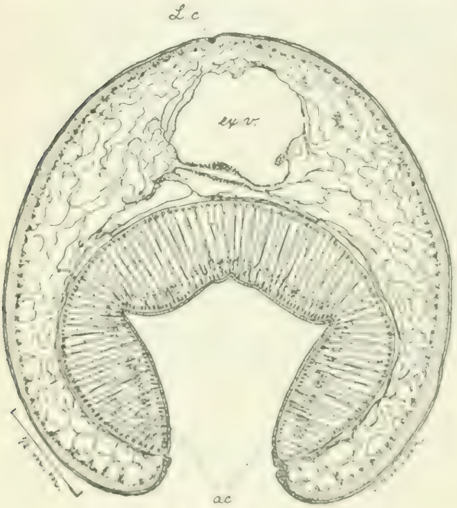

FIG. 102.

FIG. 98.—Transverse section at *c–c* figs. 94 and 95. Shows genital papilla (*g. pap.*), ductus hermaphroditicus (*d. h.*), metraterm (*va.*), pars prostatica (*p. p.*), intestinal ceca (*i.*), and surface papillæ (*s. pap.*). Enlarged. Original.

FIG. 99.—Transverse section at *d–d* figs. 94 and 95. Shows superior testis (*t.*), right vas efferens (*v. e. d.*), the uterus (*ut.*), intestines (*i.*), vitellaria (*v. g.*). Enlarged. Original.

FIG. 100.—Transverse section at *e–e* figs. 94 and 95. Shows caudal testis (*t.*), origin of right vas efferens (*v. e. d.*), uterus (*ut.*), intestines (*i*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 101.—Transverse section at *f f* figs. 94 and 95. Shows ovary (*ov.*), excretory pore (*ex. p.*), excretory vesicle (*ex. v.*), uterus (*ut.*), and intestines (*i.*). Enlarged. Original.

FIG. 102.—Transverse section at *g g* figs. 94 and 95. Shows acetabulum (*ac.*), excretory vesicle (*ex. v.*), and pore of Laurer's canal (*L. c.*). Enlarged. Original.

SUBGENERIC POSITION UNCERTAIN.

The subgeneric position of the following species is left open for the present:

- *Paramphistomum fraternum*, close to the subgenus *Paramphistomum*, very close to *P. explanatum*.
- *P. siamense*, close to subgenus *Paramphistomum*, very close to *P. explanatum*.
- *P. shipleyi*, close to *P. parvipapillatum* and *P. scoliocœlium*.
- *P. parvipapillatum*, close to *P. shipleyi* and *P. scoliocœlium*.

PARAMPHISTOMUM FRATERNUM, new species.

[Figs. 103 to 113.]

Specific diagnosis.—*Paramphistomum* (p. 73): Body 9.75 mm. long, at least 4.5 mm. in maximum breadth; grayish olive green in color (alcohol specimens); viewed ventrally, conical; viewed laterally, gourd shape; greatest diameter at junction between equatorial and caudal thirds; tapers gradually but considerable to truncated conical oral pole; caudal end bluntly rounded when viewed ventrally, beveled ventrally when viewed laterally; long axis curved moderately, concavity ventrad; dorsum arches both longitudinally and tranversely; venter concave longitudinally, convex transversely; transverse sections in general elliptical to semicircular with rounded angles. Surface with fine transverse striations, no papillæ. Genital pore apparently slightly sinistrad (due to torsion?) in suctorial zone about one-eighth of body length from oral pole; shallow genital atrium; genital papilla only slightly elevated. Acetabulum large, ventro-subterminal, 3.75 mm. long, 3.25 mm. broad, aperture 1.85 by 1.52 mm., margins projecting slightly, cavity very deep. Mouth terminal, crateriform, not papillated; oral sucker 0.96 mm. long, 0.98 mm. broad, 0.52 mm. thick, rather oval in outline; perisuctorial space narrow; esophagus about three-fourths as long as sucker; ceca pass at first laterad, then caudad to end at equator of acetabulum, about at junction of third with caudal fourth of body, the ends bending medio-caudad. Excretory pore opens on preacetabular plane (about five-ninths of body length from oral pole) and one-fifth of body length cephalad of pore of Laurer's canal; excretory vesicle crosses Laurer's canal, long, extends postovarian, beyond equator of acetabulum.

Male organs.—Testes equatorial, pre- and acetabular, zones and fields overlap, in extra-, inter-, and cecal areas, very large, lobate; vasa efferentia unite about at junction of oral and equatorial thirds of body; vas deferens highly developed; vesicula seminalis much coiled, extending about from pretesticular plane nearly to bifurcal plane; musculosa short, nearly straight, extends nearly to postsuctorial plane; prostatica much shorter than musculosa; ejaculatorius and ductus hermaphroditicus very short.

Female organs.—Ovary about at equator of acetabulum, at end of ceca, slightly dextrad; shell gland in ovarian zone, slightly sinistrad; vitellaria large, follicles large, in extra-, inter-, and cecal areas, dorsal and ventral of ceca, from slightly above postsuctorial plane to caudal margin of shell gland, hence slightly postcecal; uterus extends from shell gland a short distance cephalad, coils, then ascends a short distance, then coils and becomes very much distended by eggs, passing dorsally of testes and beneath arch of vasa efferentia, then runs in nearly straight course ventrally of vas deferens to discharge into ductus hermaphroditicus; Laurer's canal runs at first cephalo-dorsad, then dorsad to open slightly sinistrad of median line, slightly caudad of preovarial plane, about 1.72 mm. caudad of excretory pore.

Eggs.—Numerous, operculated, 120 by 67.5μ.

Type.—B.A.I. 3066.

Habitat.—In (organ? of) *Buffelus indicus.*

Source of material.—The 2 specimens forming the collection were in a bottle bearing the B.A.I. No. 3066, the label in which bore the following information:

Name *Amphistomum explanatum*. Host *Buffelus indicus*. Determined by Prof. A. Railliet. Date 1899. Presented by Prof. A. Railliet. Date 1899.

The specimens are in poor condition; one is greatly distorted, having its cephalic third bent acutely ventrad; the other also appears somewhat distorted by irregular shrinkage, but is sufficiently preserved for study, and the following description is based chiefly upon it.

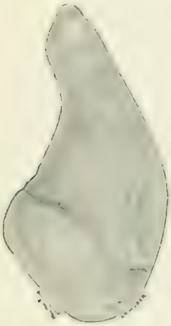
Fig. 103.

EXTERNAL CHARACTERS.

Size.—Measured in alcohol this specimen was 9.75 mm. in greatest length and 4.5 mm. in greatest transverse diameter. After embedding and sectioning, the length (calculated from the sections) was found to be 8.94 mm., the greatest transverse diameter 4.10 mm., and the greatest dorso-ventral diameter 4.4 mm. The measurement of the greatest transverse diameter was unsatisfactory on account of the distortion by flattening in that region of the worm; this measurement is therefore regarded as considerably under what it would be in the perfect specimen.

Color.—The specimens were of a grayish olive-green color.

Form (figs. 103, 104).—The greatest dorso-ventral and tranverse diameters are in about the region of junction of the middle with the caudal third of the body. From this region it tapers gradually but considerably toward the oral pole, which appears relatively sharply pointed; a short length of the terminal portion is contracted into a small truncated cone. The aboral pole remains broad and thick and rounded, and may be considered as beveled at the expense of its ventral aspect, where it presents the terminal irregularly circular acetabular aperture. The longitudinal axis of the worm is moderately curved with its concavity ventrad. The dorsum is arched both longitudinally and transversely, whereas the venter is concave longitudinally and convex from side to side; this convexity, however, is much less marked than that of the dorsum and in certain regions almost disappears. Transverse sections are in a general way elliptical in outline, but in the region where the transverse convexity of the venter almost disappears, the outline approaches that of a semicircle with rounded angles.

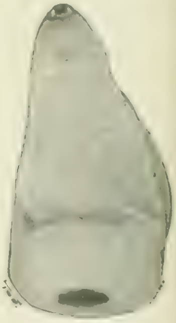
Fig. 104.

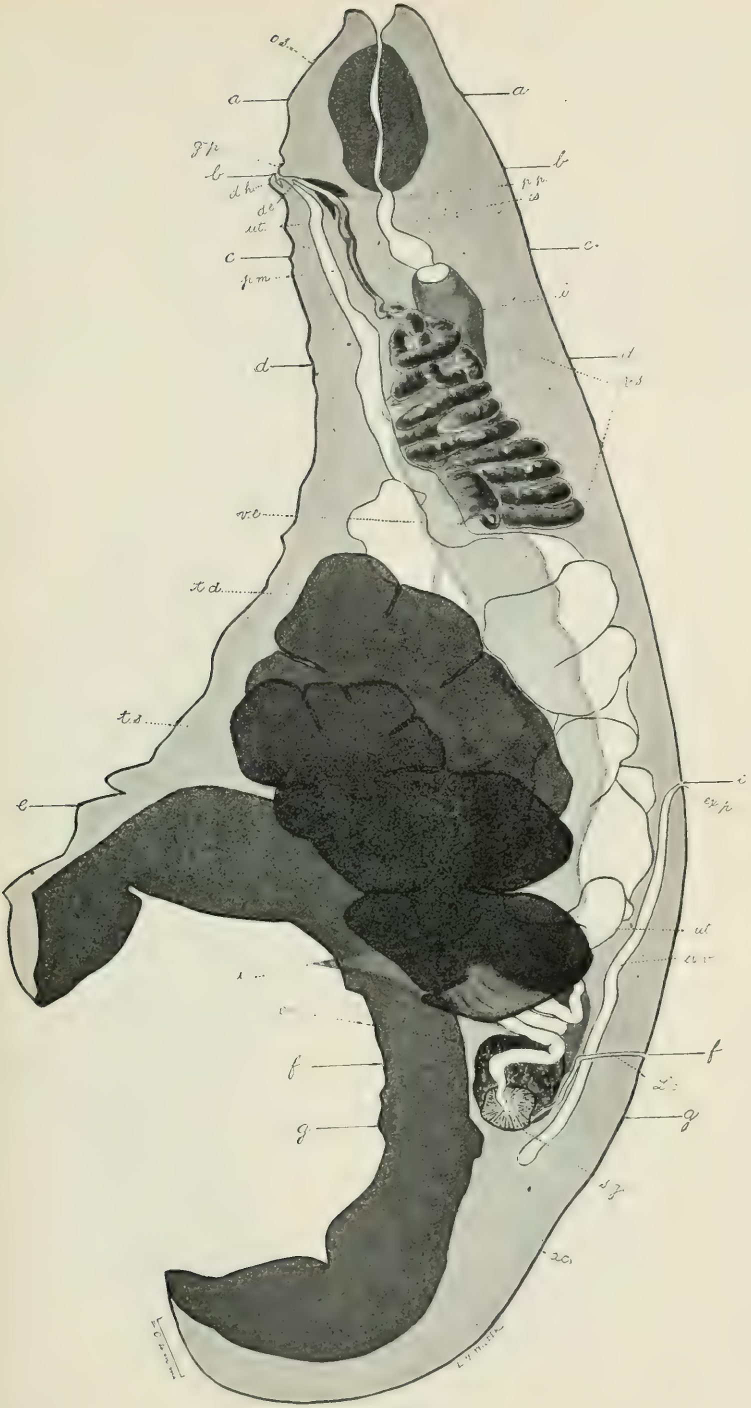
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FIG. 105.

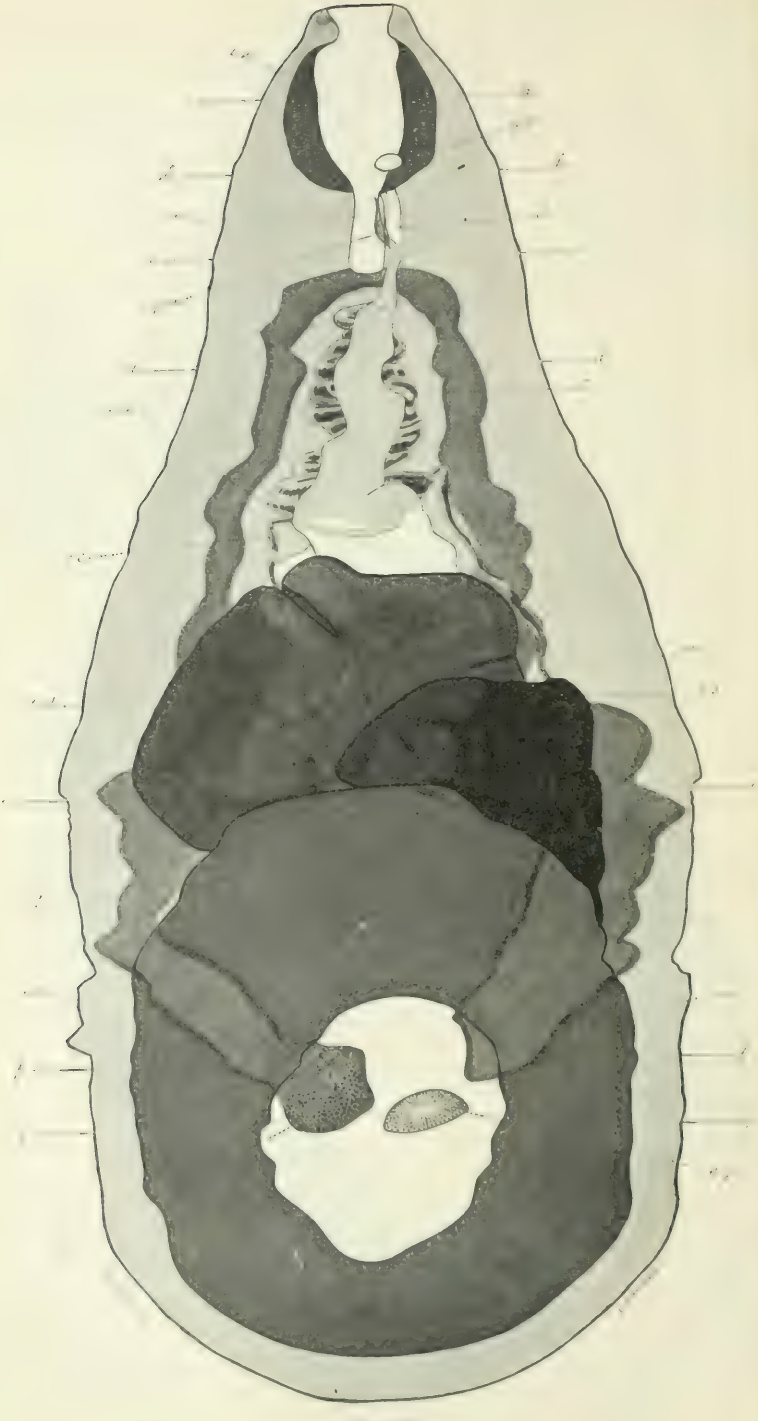


FIG. 106.

SURFACE.—The surface cuticle presents fine transverse striations, best marked near the oral extremity. There are also irregularities of the surface, which are probably due to irregularities in contraction in the process of fixing and in the preservation.

Genital pore.—On the ventral surface apparently slightly to the left of the median longitudinal line and at about one-eighth of the body length from the oral extremity is a small bulging, on the vertex of which may be noted a small (160μ) transversely elongate button-hole-like pore, the genital pore (figs. 104, 108). This pore leads into a relatively shallow chamber, which is almost entirely filled by a plump, slightly elevated, genital papilla which arises from what corresponds to the dorsal wall of the chamber. At the vertex of this papilla is an orifice, the porus hermaphroditicus, the external termination of the ductus hermaphroditicus.

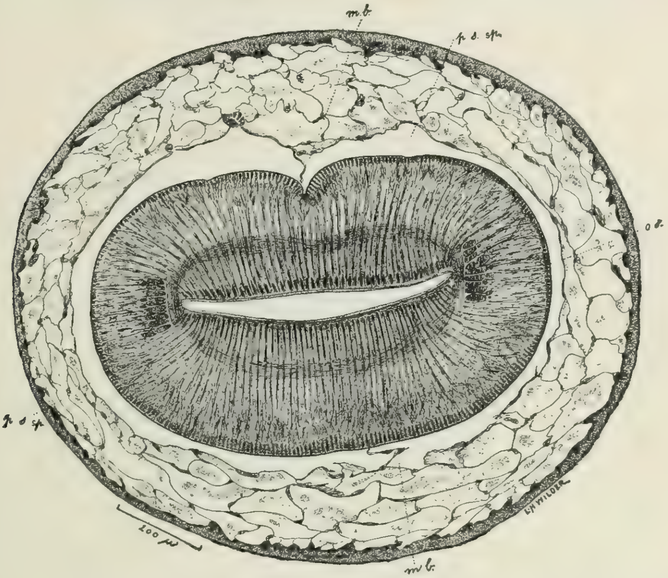


FIG. 107.

Acetabulum.—This muscular organ is in the caudal portion of the body. On account of the curvature of the longitudinal axis of the worm its aperture is tilted slightly ventrad and gives the appearance of a ventral bevel to the caudal extremity of the worm. The dome of the acetabulum (measured in projection of one sectioned specimen) was about 3.75 mm. in vertical by about 3.25 mm. in transverse diameter with an aperture about 1.85 mm. in vertical by about 1.52 mm. in transverse diameter. The margin of the acetabular aperture is formed by the slightly projecting muscular rim of the acetabulum itself, a thin, closely applied layer of the body parenchyma extending to its margin but not overlapping it, so that the aperture appears to have a narrow enclosing lip, which is readily discernible on the surface, where it is marked off from the general body surface by a shallow groove (figs. 104, 106, 112, 113).

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The cephalic extremity of the worm presents a transversely elliptical crateriform depression, the base of which presents the oral aperture. The latter leads directly into a muscular oral sucker. No papillæ were observed. The sucker is relatively small, measuring in projection about 0.96 mm. in length, with a maximum transverse diameter of 0.98 mm. and with a maximum dorso-ventral diameter (measured in median sagittal plane) of 0.52 mm. In both sagittal and frontal planes it is of an oval outline; in transverse section it has the form of an ellipse with its major axis corresponding to the transverse diameter of the worm (fig. 107). The lumen of the sucker is a dorso-ventrally narrow, transversely broad space; in transverse section in the region of its equator the form of the lumen is somewhat that of a spindle. The body of the sucker is inclosed in a narrow perisuctorial space, in which it is retained in position by attachments at both its poles and by dorsal and ventral dorso-ventral strands. The lumen is lined by a thin, smooth, cuticle-like layer. The esophagus springs from the base of the sucker, passes directly caudad for about half its length, then bending slightly, passes dorso-caudad to divide into two lateral ceca at a point slightly nearer the dorsum than the venter and about one-fifth of the body length from the oral extremity. The length of the esophagus is

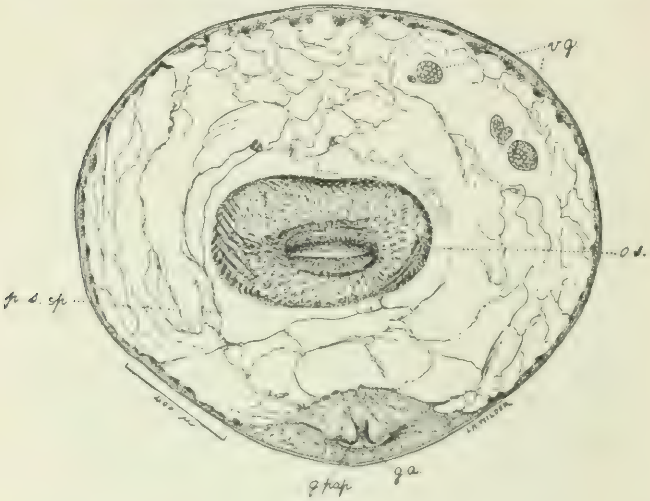


FIG. 108.

about three-fourths that of the sucker. Its anterior extremity is slightly caudad of the level of the genital pore. In the first half of its course the lumen of the esophagus maintains substantially a uniform diameter; in the second half, however, it dilates moderately but distinctly and with some abruptness. The esophagus is lined by a cuticle-like layer and is inclosed in a scant layer of sparsely scattered cells.

The intestines pass at first laterad from their point of origin from the esophagus, then, describing a curve, they pass directly caudad in fairly close proximity to the dorso-lateral aspect of the body. Their form in transverse section is very irregular and their caliber varies greatly at different levels. They terminate by cecal extremities dorsad of the acetabulum and slightly caudad of the center of the aperture, or at a level somewhat caudad of the junction of the third with the caudal fourth of the body. They are lined by an epithelial cell layer.

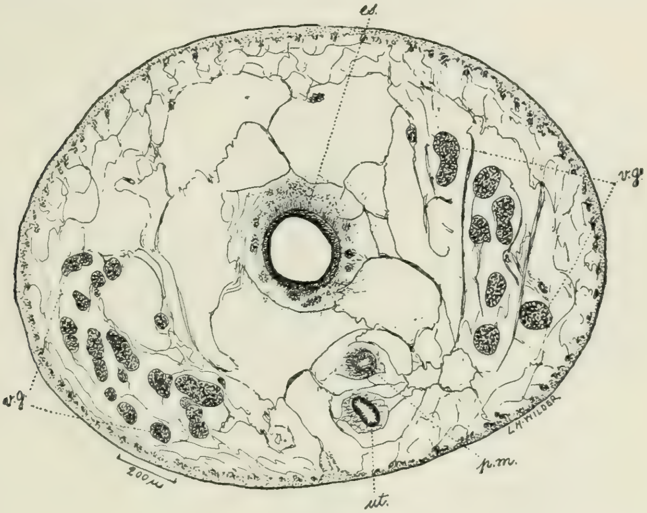


FIG. 109.

GENITAL SYSTEM.—With the exception of the vitellogene glands, the genital organs lie in the intercecal area.

Male organs.—The two testes are large irregular masses. One is a little to the right of the other and a little nearer the oral extremity, so that their zones and their fields overlap to a considerable extent (figs. 105, 106, 111). The testis from which the right vas efferens springs lies in the equatorial fifth of the worm and is a little to the right and at a slightly higher level than the testis from which the left vas efferens springs. The latter testis is therefore somewhat farther from the oral extremity and overlaps the left side of the caudal portion of the ventral aspect of the right testis. Both testes are indented by deep fissures which mark off large lobes. A vas efferens emerges from the dorsal aspect of each testis. On account of the relative positions of the testes, the right vas is much the shorter. They at first pass more or less directly cephalad; then a little above the cephalic aspect of the right testis they pass transversely inward, forming an arch beneath which the uterus passes as they unite in the formation of the vas deferens. The first portion of the vas deferens is a relatively thin walled, long, intricately and compactly coiled vesicula, the lumen of which is dilated and filled with spermatozoa (figs. 105, 106, 110). It is succeeded by a relatively short, uncoiled, and almost straight pars musculosa (figs. 105, 106, 109). The change from one to the other is quite abrupt. The musculosa has a relatively small lumen and its walls, though more muscular and thicker than that of the vesicula, are not very greatly developed. Its direction is cephalo-ventrad.

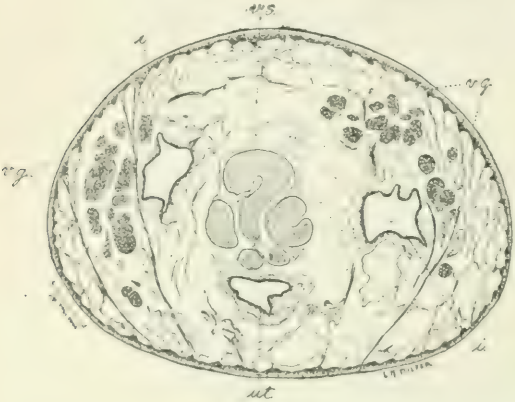


FIG. 110.

At a point in a transverse plane slightly caudal of that of the base of the sucker this duct becomes inclosed in a mass of cells and its external layer of longitudinal muscle fibers becomes much thinned; this is the beginning of the pars prostatica. The pars prostatica (fig. 105) is both absolutely and relatively quite short and is succeeded by the ductus ejaculatorius. The ductus ejaculatorius is quite short, its walls more delicate than those of the prostatica, and the change from one to the other is marked by the disappearance of the prostatic cells. At the base of the genital papillae the ductus ejaculatorius and the uterus unite to form the ductus hermaphroditicus which pierces the genital papilla and terminates at the vertex of the latter at the porus hermaphroditicus. The genital papilla and the wall of the genital atrium are inclosed in a sharply defined mass of muscular fibers (fig. 108).

Female organs.—The ovary lies in the caudal portion of the body, slightly caudad of the plane of junction of the third with the caudal fourth of the body, dorsad of the dome of the acetabulum, and a little to the right of the median sagittal plane. The oviduct springs from the caudo-mesial aspect of the ovary and passes transversely to the left toward the shell gland, near which it appears to fork into two ducts (fig. 113). The forking takes place in a transverse plane; one of the forks is Laurer's canal, the other should be regarded as the continuation of the oviduct. The latter curves slightly cephalo-ventrad to penetrate the shell gland. Laurer's canal curves around the right latero-dorsal aspect of the shell gland to gain a position dorsad of the latter, then it passes cephalo-dorsad close to the left of the excretory vesicle and opens on the dorsum a little to the left of the median line at a point in a transverse plane slightly caudad of that of the cephalic aspect of the ovary and about 1.72 mm. (or about one-fifth of the total body length) caudad of the excretory pore. The shell gland is considerably smaller than the ovary and lies a little to the left of it and of the median sagittal plane.

On its mesio-caudal aspect the shell gland is penetrated by the oviduct and on its caudal aspect near its mesial margin by the vitello-duct. These ducts unite almost at once to form the ootype. This fusiform canal pierces the shell gland obliquely cephalad and to the left, emerging from the left latero-cephalic aspect of the gland as the uterus. The uterus after emerging from the shell gland tends cephalad, curving around the dorsal aspect of the shell gland to gain the axial region of the worm. Here the uterus in its ascent cephalad describes intricate coils. It forms some loops in the space between the caudal portion of the left testis and the acetabulum, then doubles back passing underneath this testis somewhat obliquely to the left and turns cephalad in the space dorsad of this testis. It ascends in

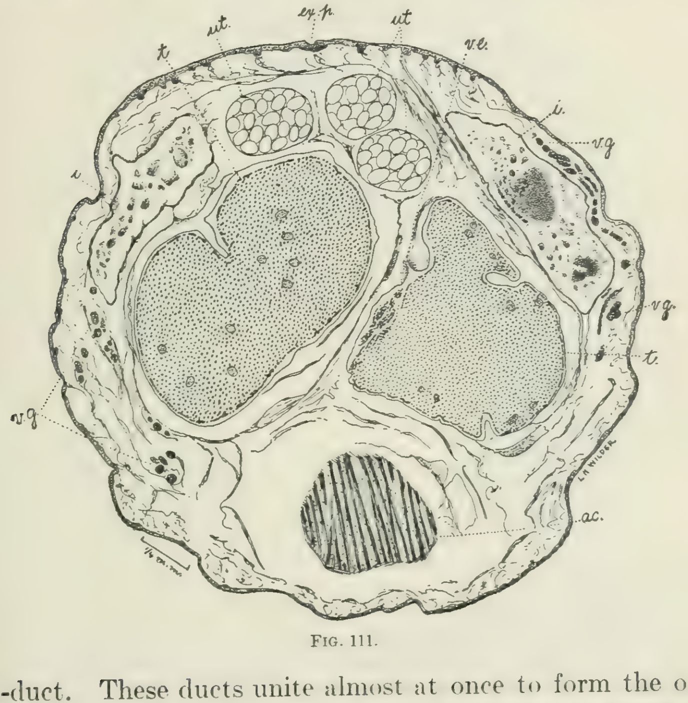


FIG. 111.

this position in an almost straight course for a considerable distance before it again begins to form coils. These coils are in the intercecal space dorsad of the testes and at first ventrad of the excretory vesicle and duct, and later between the testes and dorsum. The coils are distended with very numerous oval, operculated eggs. As the level of the cephalic aspect of the right testis is approached, the coils tend cephalo-ventrad between the vasa efferentia and gradually gain the ventral aspect of the coiled vesicula, ascending cephalad in this relation for a short distance, after which the windings cease and the eggs disappear from its lumen. It now ascends in only a slightly wavy course close to the ventral aspect of the vas deferens to unite with the ductus ejaculatorius at the base of the genital papilla in the manner already described.

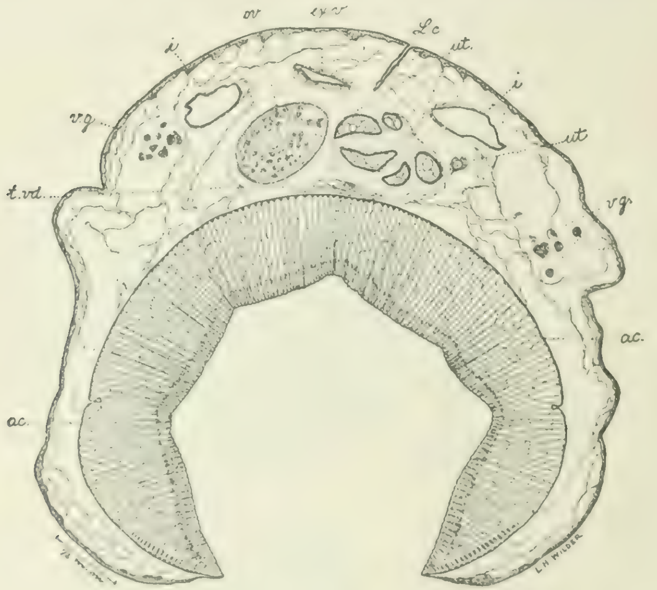


Fig. 112.

The vitellogene glands resemble those of *P. cervi*, but are not quite so highly developed. The follicles are large and prominent and occupy the extracecal areas, but extend along underneath the lateral margins toward both the ventral and dorsal median sagittal line, approaching closer, however, to the latter than to the former, and coming into relation with both ventral and dorsal aspects of the ceca. Vertically the glands extend from slightly above the level of the base of the sucker to about the level of the plane of the caudal margin of the shell gland. It will be observed, therefore, that in length they exceed that of the intestinal ceca; that is, they extend beyond the limits of the cecal zone.

A transverse vitello-duct leaves the caudal portion of each gland, passes obliquely caudo-mediad ventrally of the corresponding intestine to unite close to the dome of the acetabulum at a point in a transverse plane just above that of the cephalic aspect of the shell gland. Their union results in the formation of a common duct which passes caudo-dorsad, skirting the ventral and ventro-caudal aspect of the shell gland, finally to penetrate the latter at its caudal aspect as already described.

Eggs.—The eggs are operculated and very numerous. One of the eggs measured in section of the uterus was 120μ long by 67.5μ broad.

Excretory system.—The excretory vesicle is relatively small, being a rather long, and dorso-ventrally very narrow, almost slit-like space. Its dome is in the caudal portion of the body about midway between the acetabulum and dorsum and about 1.48 mm. (or about one-sixth of the body length) from the caudal margin of the worm. From the dome the body of the vesicle extends cephalad, gradually coming nearer the dorsum as it ascends in the interceeal area. As it ascends also it gradually narrows in both transverse and dorso-ventral diameters until it becomes reduced to a cylindrical duct about 52μ in diameter and 240μ long, with thickened walls, which opens on the dorsum at about the level of the superior margin of the acetabulum, namely, about five-ninths of the total body length caudad of the oral margin, and, as already stated, about one-fifth the body length cephalad of the opening of Laurer's canal.

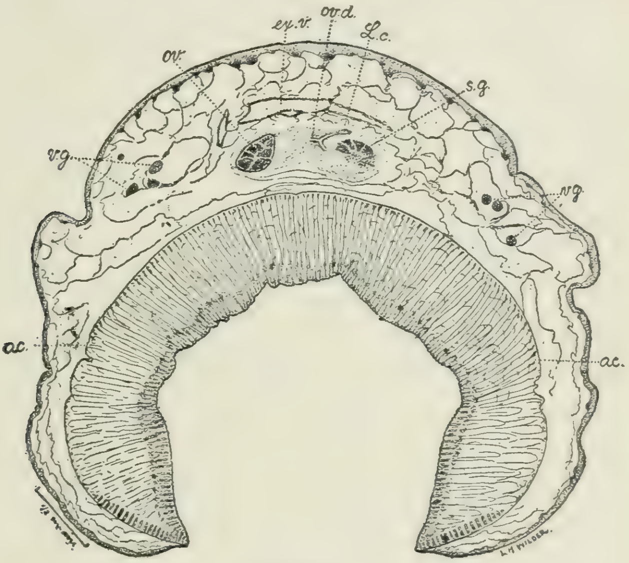


Fig. 113.

RELATION TO *P. explanatum* AS DESCRIBED BY FISCHŒDER.

Comparing our specimens with *P. explanatum*, as described by Fischœder, 1904, 454–458, figs. A, 1–3, the following differences are noticed: In *explanatum* the genital pore is immediately postbifurcal instead of suctorial; the ceca end at the cephalic margin of the acetabular aperture, instead of at the acetabular equator; excretory pore is equatorial, some distance cephalad of acetabulum, instead of on preacetabular plane; the excretory vesicle ends cephalad of acetabular equator instead of at its equator; the testes are distinctly farther cephalad, the anterior testis not reaching the acetabulum; the pars prostatica is longer instead of shorter than the pars musculosa; the ovary is at the anterior margin, instead of at the equator of acetabulum, and is distinctly and entirely in the interceeal area instead of at and caudad of end of ceca; Laurer's canal is distinctly preacetabular instead of at the equator of the acetabulum.

These differences would appear to indicate that we are dealing either with a distinct species, or a distinct subspecies, or with a rather marked case of individual variation; they are certainly more marked than the characters now being used to distinguish between some species of distomes. As it is easier to suppress a synonym than it is to disentangle anatomical and biological data of separate forms confused in one alleged species, we incline to the view (contrary to most authors) that in case of doubt the more conservative action consists in proposing a new species, hence we publish this form as such.

ILLUSTRATIONS.

FIG. 103.—Profile view. Enlarged. Original.

FIG. 104.—Ventral view of same. Enlarged. Original.

FIG. 105.—Profile projection of specimen shown in figs. 103 and 104. Shows oral sucker (*o. s.*), esophagus (*es.*), right intestinal cecum (*i.*), genital pore (*g. p.*), ductus hermaphroditicus (*d. h.*), pars prostatica (*p. p.*), pars musculosa (*p. m.*), vesicula seminalis (*v. s.*), right vas efferens (*v. e.*), right (*t. d.*) and left (*t. s.*) testis, ovary (*ov.*), shell gland (*s. g.*), uterus (*ut.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), excretory pore (*ex. p.*), and acetabulum (*ac.*); *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, planes of section. Slightly diagrammatic. Enlarged. Original.

FIG. 106.—Frontal projection of specimen shown in figs. 103 and 104. Shows oral sucker (*o. s.*), esophagus (*es.*), intestinal ceca (*i.*), ovary (*ov.*), shell gland (*s. g.*), uterus (*ut.*), the right (*t. d.*) and left (*t. s.*) testis; the vasa efferentia (*v. e.*), vesicula seminalis (*v. s.*), and pars musculosa (*p. m.*), position of genital pore (*g. p.*), and the acetabulum (*ac.*); *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, planes of section. Slightly diagrammatic. Enlarged. Original.

FIG. 107.—Transverse section at *a–a*, figs. 105 and 106. Shows form of body, form of oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), and dorsal and ventral mesenterium-like strands (*m. b.*). Enlarged. Original.

FIG. 108.—Transverse section at *b–b*, figs. 105 and 106. Shows genital atrium (*g. a.*), genital papilla (*g. pap.*), oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), and some vitelline follicles (*v. g.*). Enlarged. Original.

FIG. 109.—Transverse section at *c–c*, figs. 105 and 106. Shows pars musculosa (*p. m.*), uterus (*ut.*), esophagus (*es.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 110.—Transverse section at *d–d*, figs. 105 and 106. Shows position and relations of uterus (*ut.*), vesicula seminalis (*v. s.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 111.—Transverse section at *e–e*, figs. 105 and 106. Shows position and relations of the testes (*t.*), left vas efferens (*v. e.*), uterus (*ut.*), intestines (*i.*), superior margin of acetabulum (*ac.*), the excretory pore (*ex. p.*), and the vitellaria (*v. g.*). Enlarged. Original.

FIG. 112.—Transverse section at *f–f*, figs. 105 and 106. Shows positions and relations of ovary (*ov.*), uterus (*ut.*), excretory vesicle (*ex. v.*), Laurer's canal (*L. c.*), intestines (*i.*), vitellaria (*v. g.*), right transverse vitello-duct (*t. vd.*), and acetabulum (*ac.*). Enlarged. Original.

FIG. 113.—Transverse section at *g–g*, figs. 105 and 106. Shows origin of Laurer's canal (*L. c.*) from oviduct (*ov. d.*), the ovary (*ov.*), shell gland (*s. g.*), excretory vesicle (*ex. v.*), vitellaria (*v. g.*), and acetabulum (*ac.*). Enlarged. Original.

PARAMPHISTOMUM PARVIPAPILLATUM, new species.

[Figs. 114 to 122.]

SPECIFIC DIAGNOSIS.—*Paramphistomum* (p. 73); Body 3.1 to 3.9 mm. long by 1.8 to 2.3 mm. broad; buff color (alcohol specimens); rather oval, longitudinal outlines of lateral margins nearly straight; tapers very gradually; cephalic extremity slightly less bluntly rounded than caudal extremity; surface smooth. Genital pore postbifurcal, about one-fourth of body length from oral margin. Acetabulum subterminal, aperture about 0.5 to 0.6 mm. in diameter. Mouth terminal to ventro-subterminal (distortion?); oral sucker rather pyriform to oval; esophagus bulbous, short (?), about as long (?) as sucker, bifurcation about midway between oral margin and genital pore; ceca wavy, long, extending to or slightly caudal of equator of acetabulum. Excretory pore dorso-median, a little caudad of pore of Laurer's canal, dorsal of equator of acetabulum and not crossed by Laurer's canal; excretory vesicle dorsal of acetabulum.

Male organs: Testes occupy greater part of median field from equator to acetabulum; one caudad of the other; of irregular outline (in part artifact?); vasa efferentia unite in vas deferens; vesicula seminalis convoluted; pars musculosa short; pars prostatica short, but well developed, ductus ejaculatorius exceedingly short, unites with metraterm to form ductus hermaphroditicus, which opens on vertex of genital papilla; the latter is surrounded by an atrium into which projects a circular ridge bearing numerous exceedingly minute papillæ which also extend over on to the genital papilla: cirrus pouch absent.

Female organs: Ovary median but extending more to the left than to the right, near dorsum, immediately caudad of caudal testis and on a plane of upper margin of acetabulum; shell gland on ventro-caudal aspect of ovary; vitellaria highly developed, close to lateral margins, lateral, dorsal, and ventral of ceca, extend about from base of sucker to or slightly beyond equator of acetabulum; uterus passes from ventrally of shell gland and ovary, dorsad between ovary and caudal testis, cephalad dorsally of testes, ventrad under arch of vasa efferentia, cephalad to ductus hermaphroditicus; it is coiled, and filled with a fairly large number of eggs: Laurer's canal passes from oviduct dorso-medio-cephalad and opens in median line.

Eggs: Numerous, oval, about 135 by 67μ.

TYPE.—U.S.P.H.& M.-H.S. 9962.

HABITAT.—Reticulum of "calf" (*Bos indicus* var.) at Phrapatoom, Siam.

SOURCE OF MATERIAL.—This material was sent to us by Dr. Paul G. Woolley, who collected it in Phrapatoom, Siam, on September 22, 1906, from the reticulum of a calf (*Bos indicus* var.).

EXTERNAL CHARACTERS.

SIZE.—The specimens vary from 3.1 mm. to 3.9 mm. in length and from 1.8 to 2.3 mm. in maximum breadth.

COLOR.—Alcohol specimens are of a buff color.

FORM.—In the process of fixing and in the course of preservation the specimens appear to have undergone considerable distortion, so that from the material at hand it is difficult to more than suggest very roughly their original form (fig. 114). They are oval in outline, with lateral margins approximately parallel in the equatorial region and with extremities that are bluntly rounded. The dorsal (and perhaps also the ventral) surface is probably arched in the fresh state, but in our specimens they are marked by ridges and depressions due probably to the shrinking influence of the fixing and hardening solutions.

SURFACE.—The general surface is smooth, except for an indication of transverse wrinkles or striations. No surface papillæ are evident. The cephalic extremity is marked by the presence of a more or less circular oral aperture, and the caudal by the presence on its ventral aspect of a large subterminal acetabulum, distorted in form in most of the specimens in such a manner as to make its aperture longitudinally elliptical in outline, though one or two retained what is probably their normal circular form.

Genital pore.—The genital pore is ventro-median (figs. 114, 115, 119, 120) on a somewhat flattened rounded elevation or bulging about one-fourth the body length from the cephalic end and caudad of the bifurcation of the esophagus.

Acetabulum.—The acetabulum is distinctly subterminal in all specimens. It varies considerably in respect to form, position, and aperture, these variations being largely due, in all probability, to the distorted condition of the body. Two specimens presented circular apertures 0.5 to 0.6 mm. in diameter; four specimens presented more or less elliptical apertures, varying from 0.6 to 0.7 mm. in longitudinal and 0.17 to 0.4 mm. in transverse diameter. The rim of the acetabulum appears to project beyond the body parenchyma in a manner very like that in *Pseudodiscus collinsii*, and there is a strong suggestion of a ring around the aperture similar to that which occurs in *Ps. stanleyii* and *Ps. collinsii*. As a matter of fact, however, the apparently projecting ring is covered by a thin layer of parenchyma. The deep narrow groove which marks off this ring in *Ps. stanleyii* and *Ps. collinsii*, while strongly suggested, is not so clearly recognizable in this species (figs. 116, 122). The dome of the acetabulum measures about 126μ to 135μ in thickness.

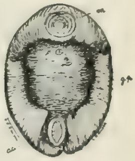


FIG. 114.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The more or less circular oral aperture gives entrance directly into a somewhat pyriform muscular oral sucker (fig. 116). In transverse section (fig. 117) the latter is elliptical in form, with its major axis in the transverse diameter of the body. Its lumen is a dorsoventrally more or less narrow, transversely fairly broad space. It is lined by a thin cuticle-like layer which appears to have disappeared from some portions of the lumen, and bears some conical papillæ of moderate size on its surface. The sucker lies in a perisuctorial space (figs. 116, 117) suggestive of a rudimentary body cavity. Dorsally of its esophageal extremity there is a transverse ganglionic cord (fig. 115). The sucker is succeeded by the esophagus. From our preparations it is impossible to determine satisfactorily its length; it is estimated, however, that it is not shorter than the sucker. It passes at first for a short distance caudad, then bends abruptly and passes almost directly dorsad, but with an inclination caudad (fig. 118), terminates, and gives origin laterally to the intestinal ceca.

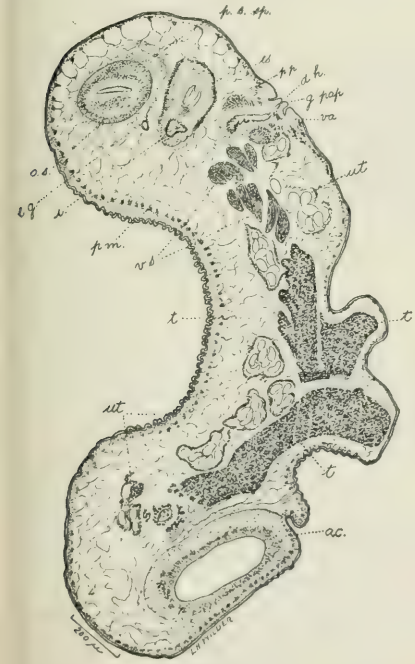


FIG. 115.

The esophagus has somewhat the form of an Indian club; its caudal half becoming, rather abruptly, very much swollen by a very great increase in thickness in its muscular layer (figs. 115, 118). The intestines, from their point of origin, pass at first in a nearly horizontally transverse plane laterad, describing in this part of their course a gentle curve nearly parallel to the dorsum. They then, rather abruptly, bend caudad and pursue a wavy path approximately parallel to the dorso-lateral aspect of the worm, terminating by blind extremities in slightly different planes at about the level of the equator of the acetabulum.

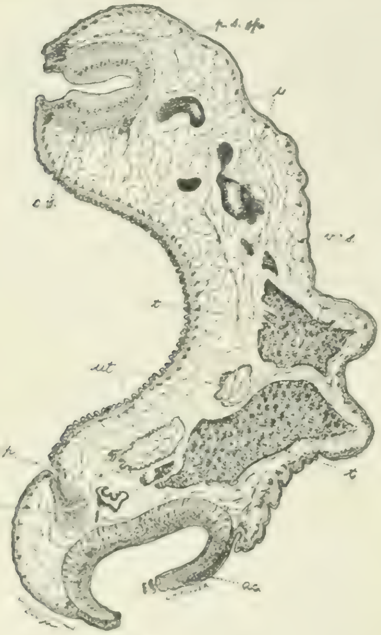


FIG. 116.

The lining of the lumen of the mouth, sucker, and esophagus appears to be a thin cuticle in anatomical continuation with that of the body surface. With the termination of the esophagus at its point of division into the intestinal ceca the cuticular lining ceases and is replaced in the intestinal ceca by a layer of epithelial cells.

GENITAL SYSTEM.—*Male organs.*—The testes occupy almost all of the interspace between the intestinal ceca from the equator (or a little anterior of this) to the acetabulum. They are placed one directly caudad of the other, with but a slight interval between. Their form is very irregular (figs. 115, 116, 121), a result, perhaps, of the shrinking and distortion of the worm as a whole, but their surface is but little indented. A vas efferens rises from each testis; that from the posterior springs apparently from the superior aspect of its right extremity and passes cephalad between the anterior testis and the right intestine; that from the anterior testis springs from its dorsal aspect, passes cephalo-centrad to unite with the left vas to form a vas deferens. The vas deferens consists of:

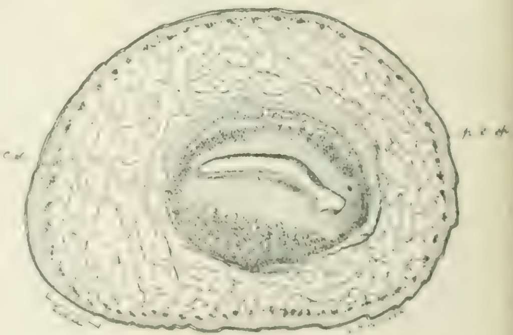


FIG. 117.

a well-developed thin-walled vesicula, which is very much coiled; a short but well-developed pars musculosa; a short but very well-defined prostatica; and a terminal excessively short ductus ejaculatorius. The latter unites with the metraterm to form a ductus hermaphroditicus. The ductus hermaphroditicus pierces the axial region of a well-developed truncated conical genital papilla. The genital papilla (figs. 115, 119, 120) projects into an atrium, which it almost fills. Embracing the genital papilla is a ring-like collar, the surface of which is beset by excessively minute papillæ (hence *parvipapillatum*), which also extend to some extent over the base of the genital papilla. External to the ring-like collar and marked off from it by a groove is another ring. This latter ring almost disappears in some positions of extrusion of the copulatory apparatus, forming a shallow crater, in the center of which is the genital pore.

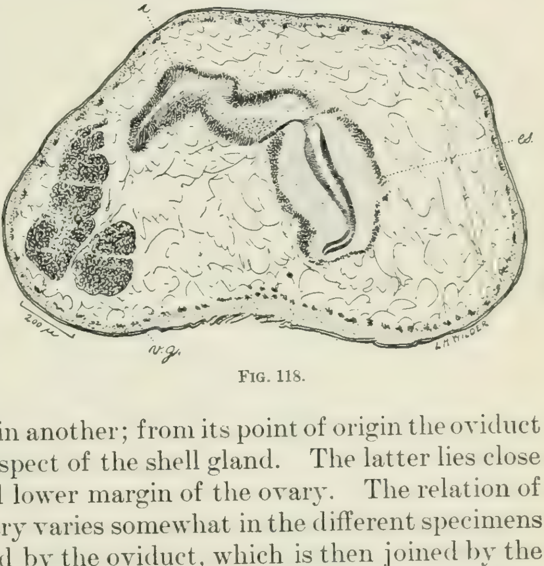


FIG. 118.

Female organs.—The ovary lies in the median line (fig. 121), close under the dorsum just caudad of the posterior testis, and at the level of the upper margin of the acetabulum. It appears somewhat elongated in the transverse diameter and compressed dorso-ventrally, hence somewhat pyriform, and extends somewhat more to the left than to the right of the median line. The point of origin of the oviduct was from the right extremity in one preparation and from the left in another; from its point of origin the oviduct passes to the opposing aspect of the shell gland. The latter lies close to the ventral aspect and lower margin of the ovary. The relation of the shell gland to the ovary varies somewhat in the different specimens studied. It is penetrated by the oviduct, which is then joined by the vitello-duct, after which the duct thus formed dilates somewhat to form the ootype; the latter is continued and emerges from the shell gland as the uterus. After emerging, the uterus passes at first caudad for a short distance, then turns, forms a loop, and passes cephalad in front of the shell gland and ovary, dorsad between the posterior testis and ovary, then cephalad between the testes and dorsum of the worm. In its progress it forms coils which are distended with eggs. At the level of the cephalic aspect of the anterior testis it takes a course ventrad beneath the arch of union of the vasa efferentia and penetrates the base of the genital papilla in company with the terminal portion of the vas deferens, with which it opens into the ductus hermaphroditicus.

The yolk glands are highly developed. They consist of numerous closely aggregated follicles in the lateral portion of the body close to the lateral margins. They inclose dorsally, ventrally, and laterally the corresponding intestinal cecum. The gland of the left side extends somewhat farther toward the median line than that of the right side, so that it comes into relation with the dorsal and the ventral aspects of the left ends of both testes. The glands extend, longitudinally, from the termination of the sucker to about the equator of the acetabulum (or slightly caudad of the level of termination of the intestinal ceca). A little above the level of the caudal termination of the yolk glands the duct of either side is given off and passes toward the middle line, ventrally of the corresponding intestinal tube, to unite with the duct of the opposite side; by the union of these a duct is formed which passes dorsad toward the shell gland, which it penetrates on its caudal aspect to join the oviduct.

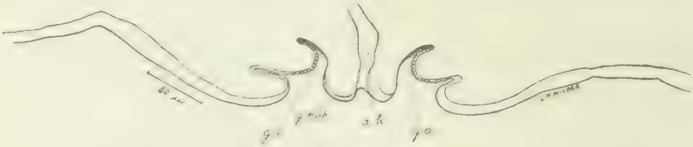


FIG. 119.

Laurer's canal leaves the oviduct about where the latter is on the point of penetrating the shell gland and passes dorso-mediad and very slightly cephalad to open on the dorsal surface about in the median line (fig. 122).

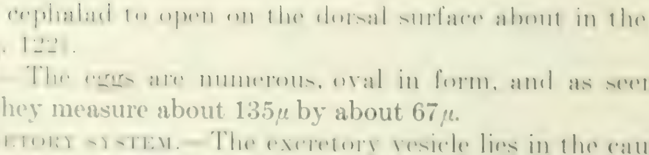


FIG. 120.

Eggs.—The eggs are numerous, oval in form, and as seen in the uterus they measure about 135μ by about 67μ.

EXCRETORY SYSTEM.—The excretory vesicle lies in the caudal portion of the body behind (dorsad of) the acetabulum. In the specimens studied it appears collapsed. From its caudo-dorsal aspect a short thick-walled duct passes dorsad to discharge through the excretory pore in the median line in a plane a little caudad of the opening of Laurer's canal and near the caudal extremity of the body. It is not crossed by Laurer's canal.

Fig. 114.—Ventral aspect: *ac.*, acetabulum; *g. p.*, genital pore; *m*, mouth. Enlarged. Original.

Fig. 115.—Sagittal section. Shows portion of oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*) containing granular coagulum, caudal portion of esophagus (*es.*), left intestinal cecum (*i.*), esophageal ganglion (*e. g.*), genital papilla (*g. pap.*), ductus hermaphroditicus (*d. h.*), pars prostatica (*p. p.*), vesicula seminalis (*v. s.*) distended with spermatozoa, metraterm (*ra.*), uterus (*ut.*) distended with eggs, testes (*t.*), and acetabulum (*ac.*). Enlarged. Original.

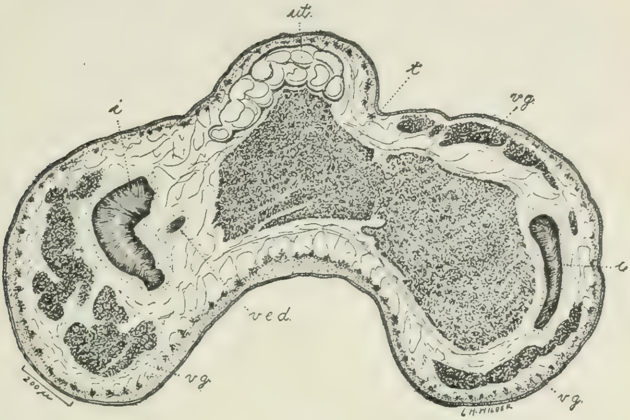


Fig. 121.

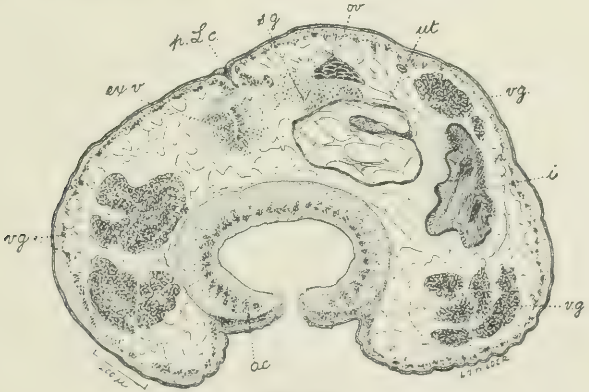


Fig. 122.

Fig. 116.—Sagittal section. Shows oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*) with granular coagulum, left intestinal cecum (*i.*), the two testes (*t.*), left vas efferens (*v. e. s.*), uterus (*ut.*), excretory vesicle (*ex. v.*), excretory pore (*ex. p.*), and acetabulum (*ac.*). Enlarged. Original.

Fig. 117.—Transverse section. Shows oral sucker (*o. s.*) and perisuctorial space (*p. s. sp.*) filled with a granular coagulum. Enlarged. Original.

Fig. 118.—Transverse section. Shows bulbous portion of esophagus (*es.*), right intestine (*i.*), vitellogene gland (*v. g.*). Enlarged. Original.

Fig. 119.—Transverse section of copulatory apparatus. Shows genital papilla (*g. pap.*), ductus hermaphroditicus (*d. h.*), genital atrium (*g. a.*), and minute papillæ on ring-like collar and base of genital papilla. Enlarged. Original.

Fig. 120.—Transverse section through copulatory apparatus to contrast with figure 119. Shows form of genital papilla (*g. pap.*) encircling papillated ring, slit-like dorsal chamber (*g. a.*), (*d. h.*) and shallow crater-like depression (ventral chamber) at vertex of genital bulging due to retraction of outer ring. Enlarged. Original.

Fig. 121.—Transverse section. Shows superior testis (*t.*), right vas efferens (*v. e. d.*), intestines (*i.*), and vitellaria (*v. g.*) and uterus (*ut.*) filled with eggs. Enlarged. Original.

Fig. 122.—Transverse section at level of pore of Laurer's canal. Shows pore of Laurer's canal (*p. L. c.*), caudal margin of ovary (*ov.*), caudal margin of shell gland (*s. g.*), uterus (*ut.*), excretory vesicle (*ex. v.*), left intestinal cecum (*i.*), vitellaria (*v. g.*) and acetabulum (*ac.*) with an apparent though not actual projecting rim. Enlarged. Original.

PARAMPHISTOMUM SHIPLEYI, new species.

[Figs. 123 to 130.]

Specific diagnosis.—*Paramphistomum* (p. 73): Body apparently somewhere between 4.5 and 7 mm. long by 2.46 mm. broad by 2.26 mm. thick; color (?); greatest breadth about at junction of equatorial and caudal thirds; tapers toward bluntly pointed oral pole, diameters at genital pore 2 mm. (transverse) and 1.98 mm (dorso-ventral). Surface with minute papillæ on oral pole. Genital pore ventro-median, about 75μ in diameter, about at junction of cephalic and equatorial thirds, very slightly postesophageal and postbifurcal, in cecal zone; at this point there is an ill-defined slightly bulging area. Acetabulum ventrosubterminal; very slightly sunken below surface, 1.15 mm. in transverse, 1.95 mm. (?) in dorso-ventral diameter, aperture about 0.39 mm. Mouth nearly terminal, with ventrosubterminal tendency, in papillated depression; oral sucker rather large, pyriform, but somewhat flattened dorso-ventrally, its oral pole projecting slightly, its lumen papillate; lies in a well defined pseudobody-cavity; esophagus acutely bent, convexity ventrad, caudal half with greatly thickened muscular wall; ceca of very irregular diameter, wavy, extend about to equator of acetabulum. Excretory pore dorso-median, caudad of Laurer's canal, somewhat caudad of equator of acetabulum; thick walled excretory canal runs from pore slightly caudo-ventrad to dorso-caudal aspect of well-developed excretory vesicle, which lies dorsad of acetabulum, extending from near anterior plane of acetabulum to caudal acetabular plane.

Male organs.—Testes large, lobate, one ventro-caudad of the other, fields nearly coincide, zones overlap, nearer venter than dorsum; vasa efferentia run cephalad at side of testes, their point of union is undetermined; vas deferens composed of: (1) coiled vesicula seminalis, which lies dorsally of (2) a pars intermedia and (3) the highly developed coiled pars musculosa, (4) pars prostatica straight, relatively short, but well developed, and separated from musculosa by a sphincter-like constriction; (5) thick walled ductus ejaculatorius; the latter opens into a slit-like space into which metraterm also discharges; from here a short duct passes ventrad to another slit-like atrium; from the latter a duct passes ventrad to open into a small atrium which opens to the exterior through the genital pore; this series of atria and canals is inclosed in a mesh of muscular fibers.

Female organs.—Ovary and shell gland dorso-caudal of and very much smaller than caudal testis, dorsal of cephalic portion of acetabulum, slightly dextral of median line; shell gland ventro-lateral of ovary; vitellaria with well developed, not numerous follicles, in extracecal areas, extend from slightly preesophageal zone to caudal end of cecal zone; uterus extends slightly caudad then cephalad, dorsally of testes, ventrally of the greater mass of pars musculosa, to open into same slit as does ductus ejaculatorius.

Eggs.—Oval, 135 by 71μ, operculated at smaller pole.

TYPE.—U.S.P.H. & M.H.S. No. 10717 (returned to Shipley).

HABITAT.—In (? stomach of) *Cervus eldi*, locality (?).

SOURCE OF MATERIAL.—The material, consisting of a series of transverse sections of one specimen, was loaned to us by Dr. A. E. Shipley. Host.—*Cervus eldi* (?), stomach.

EXTERNAL CHARACTERS.

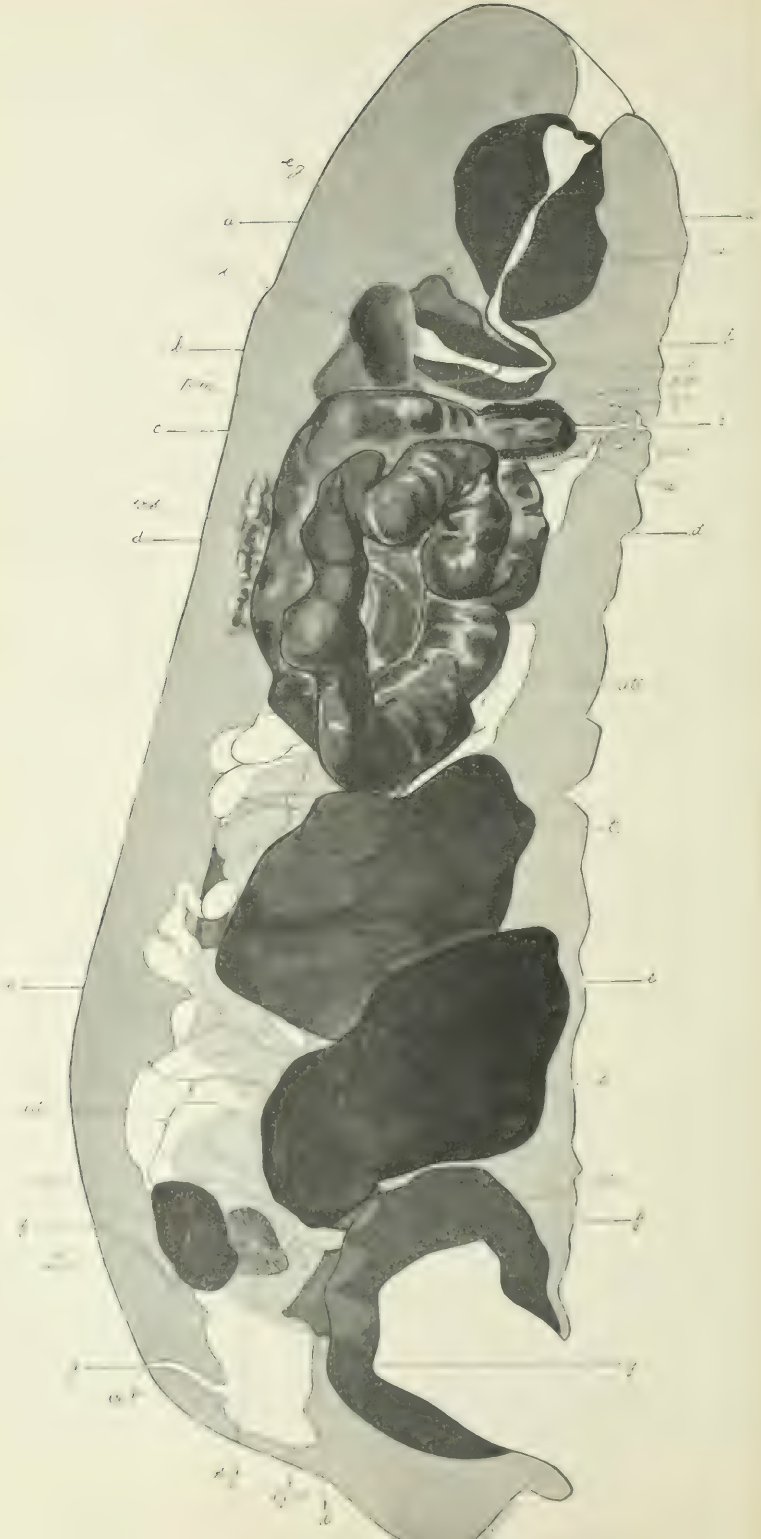
SIZE.—The specimen came to us already sectioned. We do not know the thickness of the individual sections, so can not do more than roughly estimate the length of the worm. It is probably not less than 4.5 mm. nor more than 7 mm. long, with a maximum dorso-ventral diameter of about 2.26 mm. and a maximum transverse diameter about 2.46 mm. as measured in sections.

FORM.—The worm is broadest and thickest in the regions of junction of the middle with the caudal thirds of the body length. From this region the body tapers toward the oral pole, which is bluntly pointed and at about the level of the genital pore, measures about 2 mm. in width and about 1.98 mm. in dorso-ventral diameter.

SURFACE.—The surface cuticle is unprovided with hooks or spines, but minute papillæ could be made out at the oral pole.

Genital pore.—In the ventro-median line, about at the junction of the first with the middle third of the body length, is a small orifice about 75μ in transverse diameter, the genital pore. This is in the center of a circular, ill-defined, slightly bulging area.

Acetabulum.—The acetabulum is in the caudal portion of the body with, probably, a terminal (or subterminal) aperture, though this is shown as ventro-subterminal in the diagram (fig. 123). At the level of the excretory pore the acetabulum measures about 1.15 mm. in transverse diameter with an aperture of about 0.39 mm. in the same diameter. The rim of the acetabulum, although it does not project beyond the embrace of the body parenchyma, is covered only by a relatively thin layer of it for a distance of about 255μ (fig. 130) from


eg
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the margin of the aperture. Judging from the appearance of this in section, the unsectioned specimen probably presents corresponding to it a collar-like area immediately around the acetabular aperture delimited from the general surface by a shallow, more or less well-defined groove, somewhat like but not so well defined as that in *P. siamense.*

INTERNAL ANATOMY.

Digestive tract.—The mouth is at the bottom of a circumscribed depressed area at the vertex of the cephalic extremity. It is an irregular orifice which leads directly into the lumen of a well-developed sucker. The crater-like depression of the surface is beset with digitate papillæ. In form the sucker is pyriform though somewhat flattened dorso-ventrally. Its caudal pole is broad and rounded and gives origin to the esophagus; its oral pole projects in an irregular ring-like manner beyond the parenchyma and its aperture is the mouth. The sucker is placed in a well-marked perisuctorial space (fig. 124), which is traversed dorsally and ventrally by mesenterium-like strands. The suctorial wall may be regarded as consisting of a ventral and of a dorsal muscular plate, the two, however, being continuous laterally. The muscular fibers form a dense inner and a looser meshed and relatively thicker outer zone, as seen in transverse section. The lumen is a dorso-ventrally narrow, transversely broad space which caudally becomes reduced to a small circular aperture leading into the esophagus. It is lined by a thin cuticle which is beset by small hemispherical to conical papillæ. These are more numerous and larger near the oral pole. Close to the dorso-caudal aspect of the sucker, slightly above the level of the origin of the esophagus and just without the perisuctorial space, there is a well-defined transverse nerve trunk (fig. 123) which gives off branches cephalad and caudad; these, however, can not be traced satisfactorily.

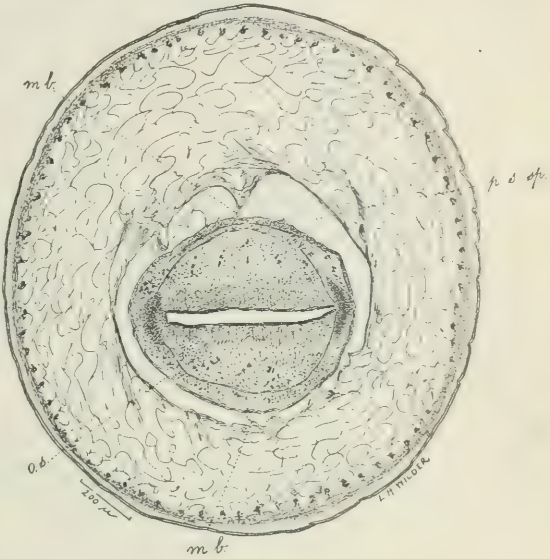

Fig. 124.

The esophagus passes at first ventro-caudad for approximately half its length, then, bending acutely, it turns dorsad, with a slight tilt cephalad, to fork into the lateral ceca at a point in a transverse plane slightly caudad of that of the base of the sucker and a little cephalad of that of the genital pore, and about or slightly more than one-third of the dorso-ventral diameter of the worm at that level from the dorsum. The muscular wall of this second portion of the esophagus is very greatly increased in thickness; the increase begins at about the point where the esophagus bends, and it augments progressively almost but apparently not quite to its caudal end. The esophageal lumen is lined by a fairly thick cuticle-like layer.

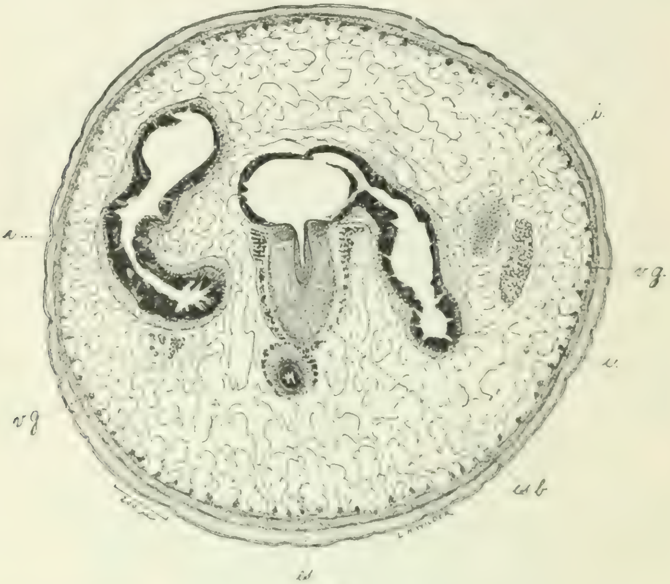

Fig. 125.

The intestinal ceca pass at first for a short distance laterad, then curve ventro-laterad, at the same time tilting caudad until they reach a point in a frontal plane somewhat dorsad of that of the bend or knee of the esophagus. Here each intestine rather abruptly curves dorsad and proceeds in this spirally, wavy course caudad until it reaches about the level of the cephalic margin of the acetabular aperture, where each intestine terminates by a cecal extremity.

The diameter of the gut varies at different points in its course, there being marked dilatations (fig. 126) succeeded by equally marked constrictions (fig. 127). The lumen of the ceca is lined by an epithelial cell layer.

Genital system.—The sexual organs, with the exception of the vitellogene glands, are disposed in the interceecal area.

Male organs.—There are two large lobate testes, one of which is caudad of the other; the cephalic portion of the caudally placed testis overlaps the right ventro-lateral aspect of the caudal portion of the cephalically placed testis. They are nearer the venter than the dorsum in a zone which exceeds somewhat in vertical diameter one-fourth the body length, in other words, comprising the third and to some extent the fourth quarter of the body. Each testis gives origin to a vas efferens, that of the caudal testis passes to the left and that of the cephalic to the right, and then each proceeds cephalad. The left vas efferens (from the caudal testis) ascends at first close to the left lateral aspect of the cephalic testis (between it and the intestine) and later, as it tends dorso-cephalad, it skirts the left lateral aspect of the coiled pars musculosa, eventually entering the coil complex of the vesicula, which is presumably formed by its union with the right vas; but this point can not be satisfactorily traced in the complexity of the coils. The course of the right vas (from the superior testis) is similar to that of the left, but is of course much shorter, and almost at once after its origin it begins to skirt the left lateral aspect of the coiled pars musculosa, eventually entering and becoming indistinguishable among the coils of the vesicula. The vesicula forms an easily distinguishable thin-walled coil complex placed close to the dorsal aspect of the coil formed by the pars musculosa. The latter is the second portion of the vas deferens, but there is intercalated, between the vesicula and

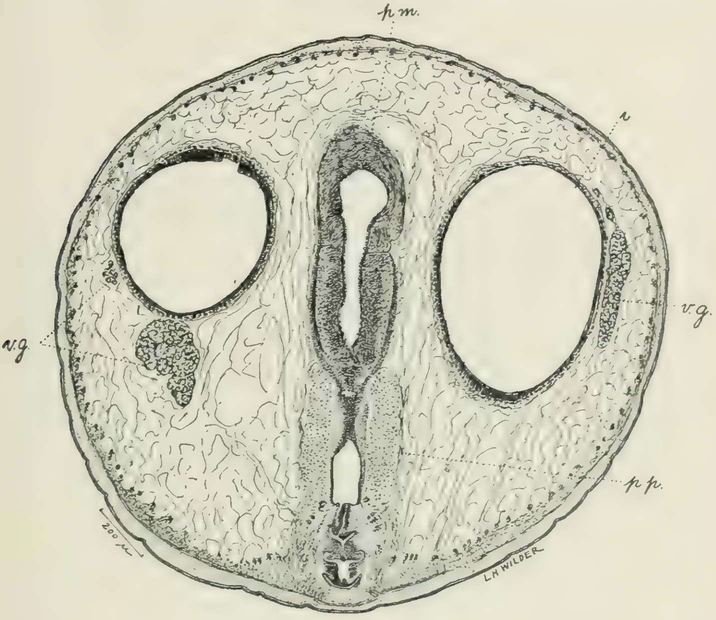

FIG. 126.

the musculosa, a short, relatively thick-walled narrow duct, which has been noted in some of the other forms and named the pars intermedia. The pars musculosa (fig. 127) is highly developed, thick, muscular walled, and very much coiled; measurements at favorable points give a diameter of about 225μ to 300μ, with a thickness of wall of about 45μ to 60μ. The caliber of the lumen of this part of the vas deferens considerably exceeds that of the vesicula. The musculosa is succeeded by a relatively short (420μ) but well-developed prostatica, a sphincter-like constriction marking the transition from one to the other. The prostatic cells are well developed and form a thick, encircling layer about the duct, the diameter of which is decidedly reduced as compared with that of the musculosa, and its wall is much thinner than that of the latter. The pars prostatica is straight and passes almost directly ventrad. In its turn the prostatica is succeeded by a thick-walled duct about 75μ in diameter and about 120μ long; this may perhaps be regarded as the ductus ejaculatorius. The ductus ejaculatorius is directed ventrad and opens with, but separate from, and just above the uterus, into a small narrow slit like space. From this space a short duct passes ventrad and may be regarded as piercing the axial region of a mushroom-like structure (figs. 123, 126) to open into another slit-like atrium somewhat larger, however, than the one into which the male and female ducts open. A duct about 30μ in diameter leads from this atrium and apparently pierces a stout conical

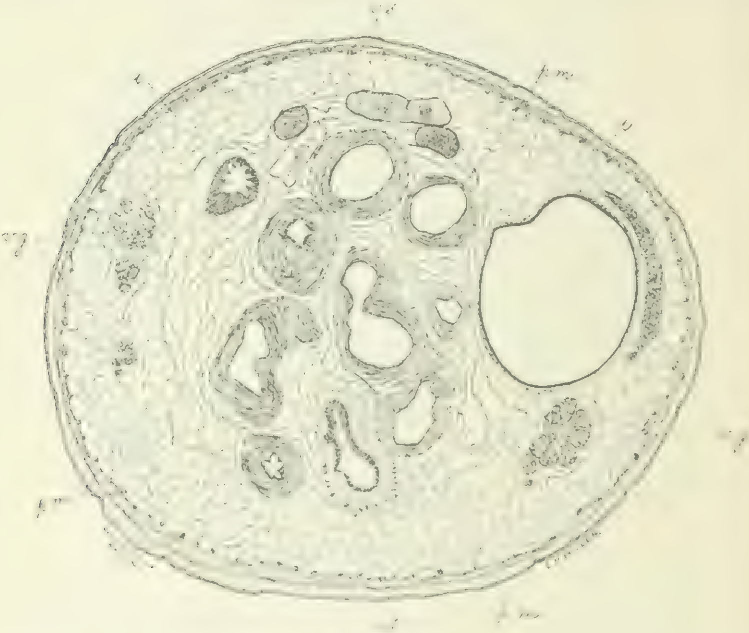

Fig. 127

papilla, which may be regarded as the genital papilla, to open into a small genital atrium which connects with the exterior by the genital pore. The series of atria and ducts connecting them, between the termination of the ductus ejaculatorius and the genital pore, which may be regarded as forming the hermaphroditic copulatory apparatus, is inclosed in a cylindrical mesh of muscular fibers, which do not, however, form such a well-defined structure as is described, for example, in *Watsonius watsoni* or *Paramphist. crassum.*

Female organs.—The ovary and the shell gland, the latter close to the right ventro-lateral aspect of the former, are in the caudal portion of the intercecal space, caudo-dorsad of the caudal testis, dorsad of the cephalic portion of the acetabulum, and immediately to the right of the median sagittal plane and the dome of the excretory vesicle. The ovary is the larger of the two glands. The oviduct takes origin from the left lateral aspect of the ovary, and at first, for a short distance, passes to the left, then again, for a short distance, it curves cephalad and to the right, after which it proceeds obliquely ventrad and to the right with a very slight tilt cephalad toward the shell gland, at the same time giving origin to Laurer's canal. This occurs in about the same transverse plane as the one in which the uterus is seen to emerge from the shell gland. The oviduct penetrates the left pole of the shell gland, in the substance of which it is joined by the common vitello-duct, the two uniting to form the ootype. The latter is directed obliquely to the right and ventro-caudad and is continued as the uterus. Laurer's canal, after parting from the oviduct, passes at

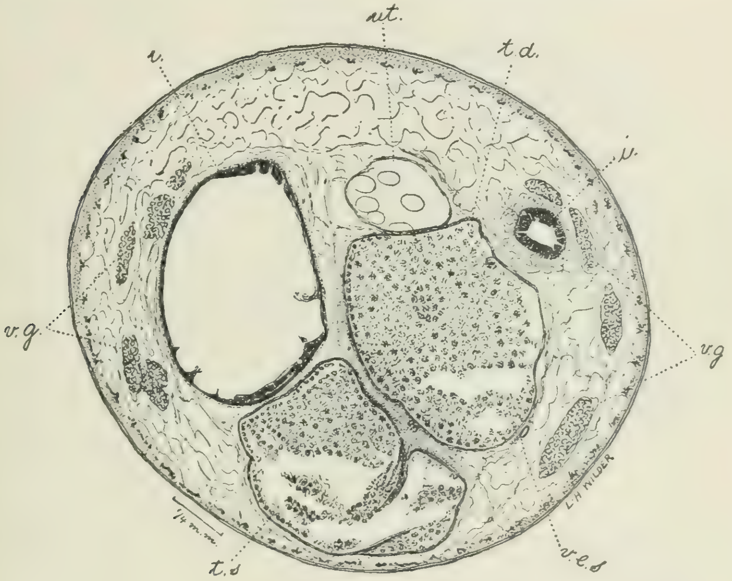

FIG. 128.

first directly caudad then with a tilt to the left and dorsad to a point slightly caudad of the level of origin of the oviduct, where it bends and passes cephalo-dorsad to open on the dorsum in about the median sagittal line at a point in a transverse plane slightly caudad of those of the cephalic margins of the ovary and the acetabulum and in about the plane of the caudal margin of the caudal testis (fig. 129).

The shell gland lies close to the right ventro-lateral aspect of the ovary; its major axis is directed obliquely from the left to the right and ventro-caudad. As already stated, it is penetrated at the left pole by the oviduct; at its left latero-cephalic aspect, it is penetrated by the common vitello-duct, the two uniting in the ootype. The uterus, which is the continuation of the ootype, emerges from the right pole of the gland and turns to the left and cephalad. It forms some coils ventrally and to the left of the shell gland and ovary, then skirts the right and dorsal aspects of the dome of the excretory vesicle to reach the left dorso-lateral aspect of the dome of this vesicle. Here, in the area between the vesicle and the left intestine, it dips caudad for a short distance, then doubles sharply cephalad to begin its ascent. It forms winding ascending coils in the intercecal space between the dorsum and the testicles. At the level of the cephalic margin of the superior (cephalic) testicle, the uterus passes ventrad, skirting the caudal aspect of the coiled vas deferens, after having first, however, ascended a short distance between this coil and the dorsum. On reaching the ventral aspect of the coil, it ascends in close relation to it and with only slight and few windings which eventually cease altogether. It finally comes in close relation to the caudal aspect of the

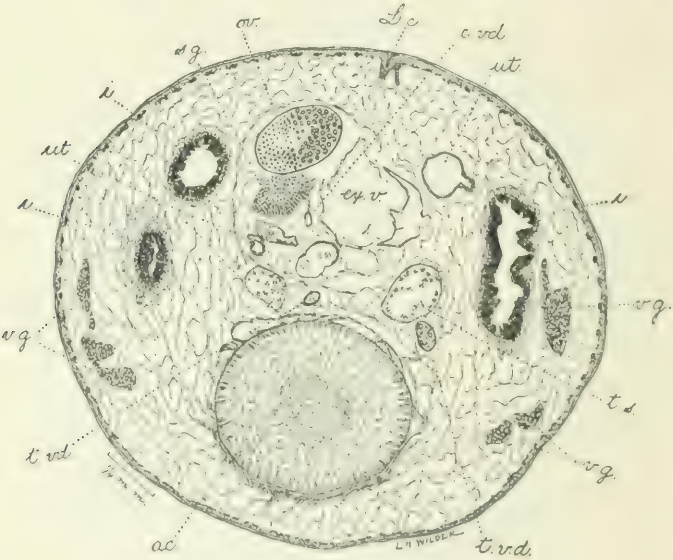

FIG. 129.

jaculatory duct, opening, as has already been stated, immediately audad of the latter into a small slit-like chamber. The first portion f the uterus contains a considerable number of yolk cells; the coils hich are between the testicles and the dorsum are distended to a ariable degree with eggs, among which in the more proximal coils nay also be seen some clumps of spermatozoa. The eggs are oval n form with a small operculum at the more pointed end and measure bout 135μ by 71μ.

The vitellogene glands, consisting of well-developed but not numerous follicles, are disposed in the extra-cecal areas (between the intesine and lateral body wall), extending vertically from a little cephalad of the level of the esophageal fork—slightly cephalad of the base of the oral sucker to or very slightly caudad of the level of the cecal ends of the gut. At about the level of the cephalic margin of the acetabulum a duct leaves each gland and passes obliquely inward and caudad, ventrally of the corresponding intestine, then bends dorsad. The two transverse ducts unite in about the median sagittal plane at a point close to the acetabulum and close to the right ventro-lateral aspect of the dome of the excretory vesicle in about the same transverse plane as that in which Laurer's canal takes its departure from the oviduct. From their point of union the common vitello-duct takes its departure; it passes obliquely dorsad and to the right with a slight tilt cephalad in the direction of the shell gland which it penetrates at its left ventro-cephalic aspect and in the substance of which it unites with the oviduct. Both the transverse and common vitello-ducts contain yolk cells, but the ducts are not notably distended and no vitelline reservoir is distinguishable.

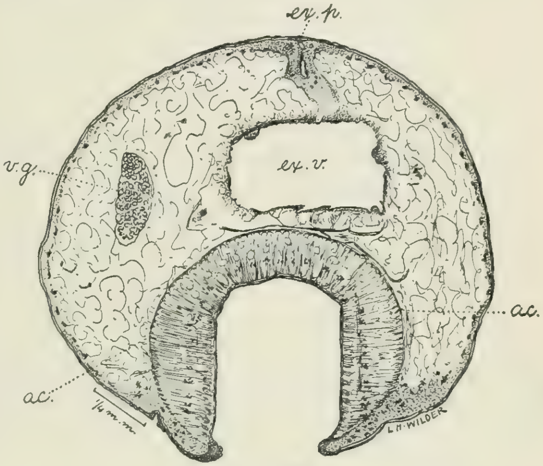

FIG. 130.

EXCRETORY SYSTEM.—A well-developed excretory vesicle is placed dorsally of the acetabulum between the latter and the dorsum. The dome of the vesicle reaches cephalad to a plane only a little caudad of that of the superior margin of the acetabulum. Caudad the vesicle extends to about the level of the caudal margin of the acetabular aperture. From the dorso-caudal aspect of the vesicle a duct takes origin; this duct passes dorso-cephalad to open in about the median sagittal line of the dorsum (fig. 130) at a point in a transverse plane passing through about the middle of the acetabular aperture and about one-ninth the body length caudad of the opening of Laurer's canal. There is no crossing of Laurer's canal and vesicle, the former lying altogether dorsally of the dome of the latter. The excretory duct is thick walled and lined with a cuticular layer in anatomical continuity with that of the surface.

RELATION TO OTHER SPECIES.

P. shipleyi appears most closely related to *P. parvipapillatum* and *P. scoliocalium*, from both of which it differs in the somewhat greater complexity of its copulatory apparatus, which is characterized by the presence of a relatively thick ring-like partition separating the ventral chamber of the genital atrium from the dorsal chamber, the two chambers being connected by a short, narrow duct. In *P. parvipapillatum* there is no partition between ventral and dorsal chambers, the line of demarcation being a fold or groove which may be (with a certain degree of evagination of the genital papilla) almost obliterated. Besides this, however, the genital atrium and base of the genital papilla of *P. parvipapillatum* are beset by minute papillæ. In *P. scoliocalium* the genital atrium forms one undivided chamber.

ILLUSTRATIONS.

FIG. 123.—Diagrammatic sagittal projection.[a] *ac.*, acetabulum; *es.*, esophagus; *e. g.*, esophageal ganglion; *ex. p.*, excretory pore; *ex. v.*, excretory vesicle; *g. a.*, dorsal chamber; *g. a. c.*, ventral chamber of genital atrium; *i.*, intestines; *L. c.*, Laurer's canal; *o. s.*, oral sucker; *ov.*, ovary; *p. m.*, pars musculosa; *p. p.*, pars prostatica; *s. g.*, shell gland; *t.*, testes; *ut.*, uterus; *v. s.*, vesicula seminalis; *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, *g–g*, planes of section. Enlarged. Original.

FIG. 124.—Transverse section at *a–a*, fig. 123. Shows oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), and mesenterium-like strands (*m. b.*). Enlarged. Original.

FIG. 125. Transverse section at *b–b*, fig. 123. Shows esophagus (*es.*), esophageal bulb (*es. b.*), intestinal ceca (*i.*), vitellogene follicles (*v. g.*). Enlarged. Original.

FIG. 126.—Transverse section at *c–c*, Fig. 123. Shows terminal portion of pars musculosa (*p. m.*), the pars prostatica (*p. p.*), terminal copulatory apparatus, intestinal ceca (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

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[a] The vertical measurements are not in accurate proportion to the dorso-ventral diameters and the dorsal line is hypothetical. It is based on a series of transverse sections, the thickness of which was not known and could not be determined accurately.

Fig. 127. -Transverse section at *d–d*, fig. 123. Shows uterus (*ut.*), oils of pars musculosa (*p. m.*), the vesicula seminalis (*v. s.*), intesinal ceca (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 128.—Transverse section at *e–e*, fig. 123, through overlapping ortions of the testes; *t. d.*, cephalic testis; *t. s.*, caudal testis; *v. e. s.*, ft vas efferens from caudal testis; *i.*, intestines; *ut.*, uterus; *v. g.*, itellaria. Enlarged. Original.

Fig. 129.—Transverse section at *f–f*, fig. 123. To show pore of aurer's canal (*L. c.*), ovary (*ov.*), shell gland (*s. g.*), uterus (*ut.*), xcretory vesicle (*ex. v.*), caudal testis (*t. s.*), right and left transverse itello-ducts (*t. vd.*), intestinal ceca (*i.*), common vittello-duct (*c. vd.*), cetabulum (*ac.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 130.—Transverse section at *g–g*, fig. 123 (level of excretory ore). *Ex. p.*, excretory pore; *ex. v.*, excretory vesicle; *v. g.*, vitelogene gland (right); *ac.*, acetabulum. Enlarged. Original.

PARAMPHISTOMUM SIAMENSE, new species.

[Figs. 131 to 136.]

Specific diagnosis.—*Paramphistomum* (p. 73): Body 6 to 9 mm. long by 4 mm. road; rather cornucopia-like in form, greatest transverse diameter just caudad of quator; greatest dorso-ventral diameter about at cephalic margin of aperture of cetabulum; tapers at first moderately then more rapidly to a bluntly pointed oral xtremity; caudal extremity bluntly rounded; longitudinal outline of lateral margins decidedly convex; anterior half of body, especially, somewhat compressed dorso-ventrally, venter somewhat concave (to some extent, at least, artifact), dorsum arched (convex) in both axes. Surface smooth, except for slight transverse ridge-like striations and a few scattered, small ventral papillæ. Genital pore ventro-median, about one-sixth of length of body from oral end and at zone of intestinal bifurcation. Acetabulum very large, 3.5 to 5 mm., in press preparation 2.6 mm. in vertical diameter, in sagittal section anatomically terminal, but because of curving of body appears to open ventro-caudad subterminally; aperture 1.2 to 1.3 mm. in longitudinal and 1.3 to 1.4 mm. in transverse diameter, directed ventro-caudad, sunken slightly below surface of worm. Mouth terminal, leads directly into globular oral sucker the lumen of which is without papillæ; esophagus somewhat shorter than sucker; intestinal ceca long, extending about to equator of acetabulum. Excretory pore dorso-median, cephalad of pore of Laurer's canal, 2.35 mm. cephalad of caudal extremity in a sagittal section, and about on plane of cephalic margin of acetabulum; excretory vesicle dorsal of acetabulum, long, extending from near cephalic margin of acetabulum to near caudal end of body.

Male organs: Testes situated in axial region of equatorial third of body, one dorso-caudad of the other; vasa efferentia arise on cephalic aspect of testes, vas deferens with much coiled vesicula, continued as musculosa to near base of genital papilla, where it changes to pars prostatica; short ductus ejaculatorius in papilla, joins with metraterm to form ductus hermaphroditicus, which opens at vertex of papilla, the latter projecting into a shallow atrium.

Female organs: Ovary somewhat pyriform, slightly lateral of median line, dorsal of equator of acetabulum; shell gland somewhat globular, about on same transverse plane but a little median of ovary; vitellaria with closely aggregated follicles, in lateral region, extending caudad from base of sucker to slightly beyond intestinal ceca; uterus extends cephalad from shell gland, coils ventrally of shell gland and ovary, passes cephalad dorsally of testes, beneath arch of vasa efferentia, then cephaloventrad to genital papilla; it is very well developed and nearly fills space between ceca; Laurer's canal skirts right side of excretory vesicle and opens slightly dextral of dorso-median line, caudad of excretory pore.

Eggs: Rather numerous, 120μ in length as measured in sections of uterus.

TYPE.—U.S.P.H. & M.-H.S. 9970.

HABITAT.—Bile ducts of a calf (*Bos indicus* var.) in Phrapatoom, Siam.

SOURCE OF MATERIAL.—This parasite was sent from Phrapatoom, Siam, by Dr. P. G. Wooley, who obtained it from the common bile ducts of a "calf" (variety of *Bos indicus*).

EXTERNAL CHARACTERS.

SIZE.—One specimen measured in glycerin alcohol was about 6 mm. long by about 4 mm. in greatest width; another measured on the slide as a press preparation was 9 mm. long.

FORM.—In form (fig. 131) the worm resembles a Sicilian fisherman's cap or a cornucopia. The caudal extremity is large, formed by the acetabulum, the aperture of which is directed ventro-caudad. The body tapers toward the cephalic extremity which is bluntly pointed and pierced by the mouth; the latter may be directed slightly forward (ventrad). The body of the animal appears somewhat compressed dorso-ventrally. The venter appears slightly excavated, due perhaps to the contraction incident to fixing. The dorsal surface is arched from side to side, and the longitudinal axis of the body forms a curve with convexity dorsad.

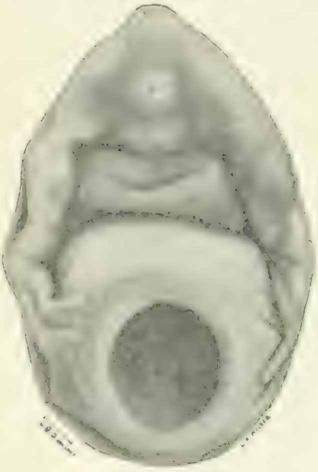

FIG. 131.

SURFACE.—The surface cuticle is smooth, except for a few slight transverse striations and a few small scattered ventral papillæ near the region of the genital pore.

Genital pore.—In the ventro-median line, about one-sixth the length of the body from the oral extremity, is a slight circumscribed bulging, in the center of which is the genital pore.

Acetabulum.—The acetabulum is large and occupies the caudal portion of the body. In three press preparations it measured 3.5, 4, and 5 mm. in diameter respectively; in a sagittal section it measured 2.6 mm. in vertical diameter, with an aperture 1.23 mm. in the same diameter. In three alcohol specimens the aperture, which is directed downward and forward (ventro-caudad), measured from 1.2 to 2 mm. in longitudinal diameter and 1.3 to 1.4 mm. in transverse diameter.

INTERNAL ANATOMY.

Digestive tract.—The mouth, which pierces the bluntly pointed cephalic extremity, leads directly into the sucker. The latter is muscular and globular in form (figs. 132, 133, 135) and is inclosed in a well-defined space, being held in place by its attachments at its oral and basal poles and by mesenterium-like strands. Its lumen, somewhat spindle-shaped and without papillæ, leads into a short esophagus. Close to the dorso-caudal aspect of the sucker there is a transverse ganglionic cord. The esophagus, passing from its origin, appears to describe a U-shaped curve, with the base of the U ventrad, and then divides into two intestinal ceca. The intestinal ceca pass latero-ventrad from their point of origin and after approaching the lateral margin, from which they are separated by the vitelline glands, they change their course caudad. They terminate by blind extremities at about the level of the equator of the acetabulum. The cecal end of the right intestine extends a little farther caudad than that of the left. In transverse section they are of irregular, variable outline, and of considerable and variable caliber in the same specimen. The lumen of the sucker and esophagus is lined by a thin layer of cuticle which ceases abruptly at the esophageal fork. The intestinal ceca are lined by a layer of epithelium.

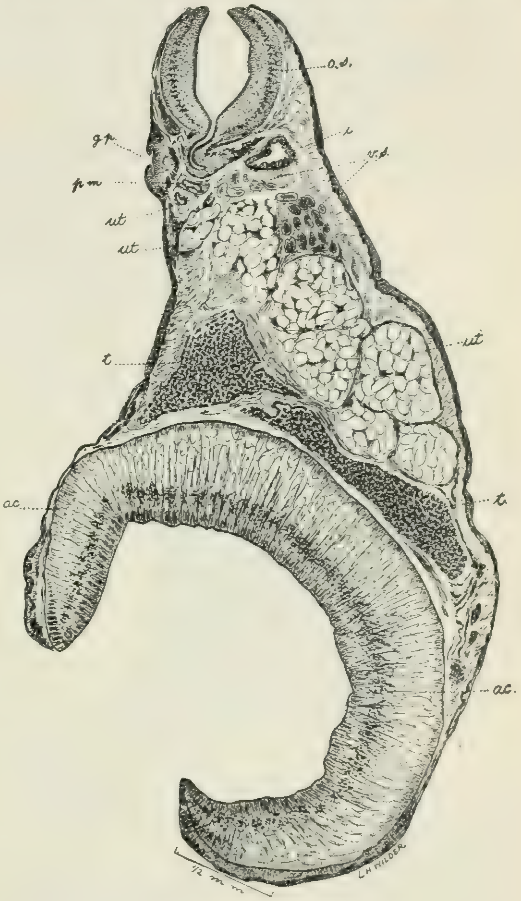

Fig. 132.

Genital system.—*Male organs.*—The testes (fig. 132) are placed one dorso-caudad or latero-caudad of the other in the axial region of the equatorial third of the body. They appear crowded together, so that the contiguous portions may overlap. In one of the specimens the degree of this overlapping was very great, whereas in another there was none at all, the testicular zones being separate, though contiguous.

The form of the testes is irregular and varies in the different specimens; this variation is probably due partly to a difference in the degree to which the uterus is filled with eggs and partly to the degree of general contraction in the fixing and hardening processes. As seen in sections, their surface is more or less indented. There appears also to be considerable variation in their position in relation to the acetabulum. In some specimens the posterior testis is above (cephalad of) the upper margin of the acetabulum; in others (figs. 132, 136) it extends to the equator of the acetabulum, the caudal aspect of the anterior testis appearing to rest on the upper margin of the acetabulum.

In neither of two series of transverse sections could the two vasa efferentia be followed for quite their entire extent, although in one of them only a very small portion of their course was not observed. Completing this portion from the other series, the following results were obtained:

A vas efferens rises from each testis; that from the right (or caudal) testis springs from the superior (cephalic) margin of the right lateral aspect and passes cephalad close to the right lateral aspect of the superior (or left) testis.

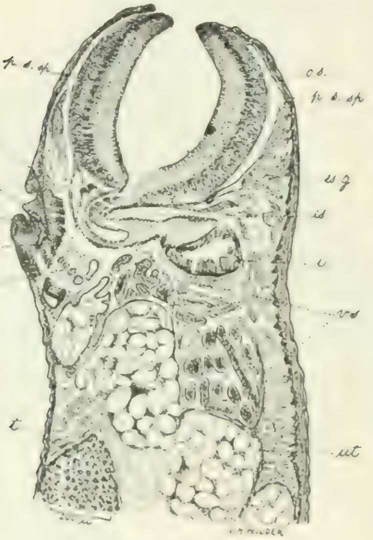

FIG. 133.

The vas efferens of the left (or superior) testis springs from the cephalic aspect of the latter and passes mediad and slightly caudad to unite with the right vas efferens to form the vas deferens. The latter is directed dorsad and almost at once dilates to form the much-coiled thin-walled vesicula. The latter passes ventrad, its wall becomes thick and its lumen becomes contracted (pars musculosa); as this latter approaches the base of the genital papilla it becomes surrounded by a large mass of cells (pars prostatica) which disappear as the duct enters the papilla. The exceedingly short terminal portion or ductus ejaculatorius joins with the metraterm in a common canal, the ductus hermaphroditicus, which opens at the vertex of the genital papilla (fig. 132).

Female organs.—The ovary (fig. 136) lies a little to the left of the edian line in the caudal portion of the body, dorsally of and in a lane passing through about the equator of the acetabulum. It is omewhat pear-shaped, with its larger pole to the right. From this xtremity the oviduct arises and passes to the shell gland. The hell gland is close to and a little to the right of the ovary and in bout the same transverse plane. It is somewhat globular, with a iameter about equal to the ventro-dorsal diameter of the broader nd of the ovary. It is pierced on its left aspect by the oviduct and n its caudal aspect by the vitello-duct which joins the former; the duct formed by their union is directed obliquely cephalad and to the right dilating to form the ootype, beyond which it is continued as the uterus. The uterus emerges from the cephalic or ventro-cephalic aspect of the shell gland and proceeds cephalad, first forming some coils ventrally of the shell gland and ovary. In its course cephalad it passes dorsally of the testes, and its coils, with lumen distended with eggs, fill the space between the intestinal ceca. Above the level of the anterior testis it forms coils beneath the vas deferens and passes ventrad toward the genital papilla, and opening, as already mentioned, into a short duct common with it and the male canal. The genital pore opens on the ventral surface at the level or slightly

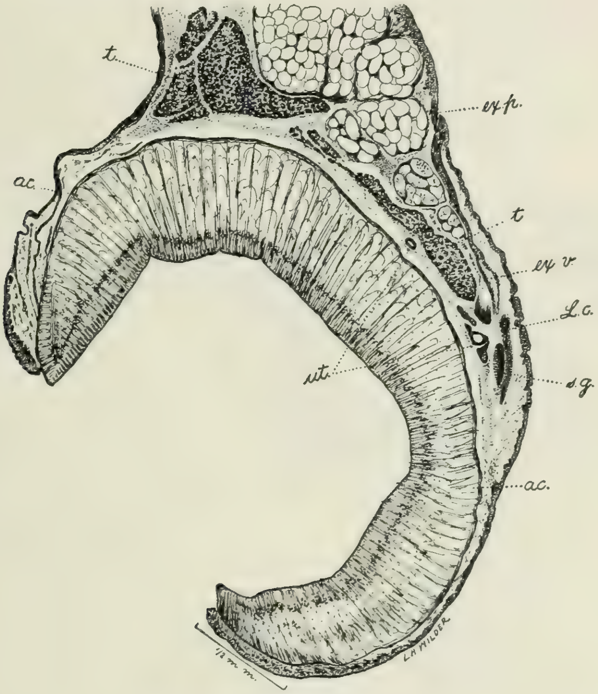

Fig. 134.

cephalad of the esophageal fork. It leads into a shallow atrium, the dorsal wall of which is formed by a low conical papilla at the vertex of which is the opening (porus hermaphroditicus) of the short duct (ductus hermaphroditicus) into which, as has been noted, the male and female canals open.

The vitellogene glands consist of well-developed follicles (of the type of *P. cervi*), more or less closely aggregated close underneath the lateral body walls. They are found not only laterally, but also dorsally and ventrally of the ceca. They begin about at the level of the base of the sucker and extend into the caudal portion of the body to a point a little beyond the termination of the intestinal ceca. A duct leaves each gland a little below the level of the upper margin of the acetabulum and passes ventrad of the corresponding intestine toward the shell gland, near the ventro-caudal aspect of which they unite to form a dilated reservoir (fig. 136). From this reservoir a slender duct arises and pierces the caudal aspect of the shell gland to join the oviduct.

Laurer's canal springs from the oviduct just as the latter is about to enter the shell gland. It describes a slight curve to the right around the corresponding margin of the excretory vesicle, to open on the dorsal surface a little below the excretory pore, slightly to the right of the median line.

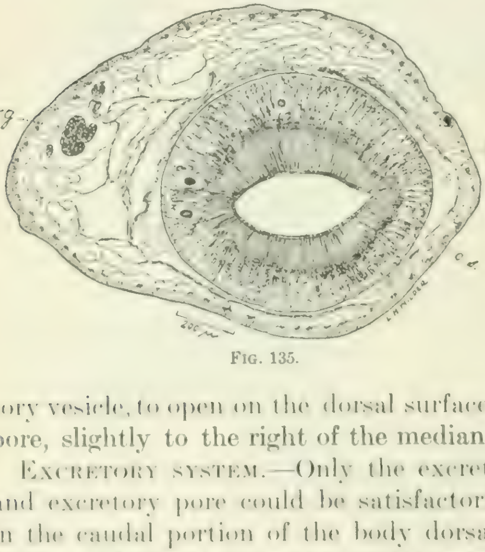

FIG. 135.

EXCRETORY SYSTEM.—Only the excretory vesicle, terminal canal and excretory pore could be satisfactorily traced. The vesicle lies in the caudal portion of the body dorsally of the acetabulum. Its fundus extends to near the caudal extremity of the worm, and its body extends cephalad close under the dorsum to the level of the upper margin of the acetabulum, at which it terminates by a short duct which opens in the dorso-median line (fig. 134) at the excretory pore, and about 2.35 mm. from caudal extremity, measured in sagittal section.

RELATIONS TO OTHER SPECIES.

This species resembles *Paramphist. cervi* and *P. fraternum*. It differs from *P. cervi* in the form of the body, that of *P. cervi* being more slender and more elongate; in the size of acetabulum, that of *P. cervi* being definitely smaller; in the position of the genital pore, tha of *P. cervi* being relatively farther caudad, about one-third of the body length from the oral margin and at or caudad of the esophageal fork, whereas in this species the genital pore is only about one-sixth the body length from the oral margin at or cephalad of the esophageal fork—that is, relatively much nearer the level of the base of the sucker than is the genital pore of *P. cervi.*

From *P. fraternum* also it differs in the form of the body, that of *P. fraternum* being markedly less flattened and its cephalic third decidedly more slender and more nearly conical; in the size of the acetabulum, that of *P. fraternum* being relatively smaller; in the position of the genital pore, that of *P. fraternum* being proportionately nearer the oral extremity, namely, about one-eighth the body length from this extremity and at or slightly cephalad of the level of the base of the sucker.

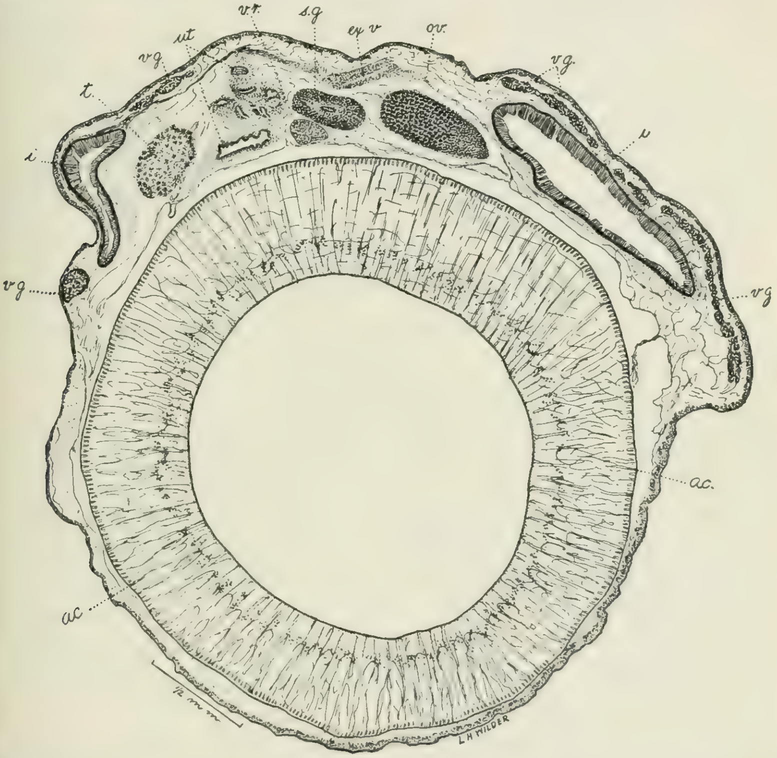

FIG. 136.

ILLUSTRATIONS.

FIG. 131.—Ventral aspect. Enlarged. Original.

FIG. 132.—Sagittal section. Shows oral sucker (*o. s.*), section of right intestinal cecum (*i.*), the two testes (*t.*), the vesicula seminalis (*v. s.*), the pars musculosa (*p. m.*), the genital pore (*g. p.*), the uterus (*ut.*), and the acetabulum (*ac.*). Enlarged. Original.

FIG. 133.—Sagittal section of oral extremity. Shows oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), esophagus (*es.*), section of right intestinal cecum (*i.*), the vesicula seminalis (*v. s.*), pars musculosa (*p. m.*), ductus ejaculatorius (*d. e.*), ductus hermaphroditicus (*d. h.*), genital papilla (*g. pap.*), the metraterm (*va.*), uterus distended with eggs (*ut.*), and cephalic portion of superior testis (*t.*). Enlarged. Original.

FIG. 134.—Sagittal section of caudal extremity. Shows acetabulum (*ac.*), the testes (*t.*), excretory vesicle (*ex. v.*), excretory pore (*ex. p.*), Laurer's canal (*L. c.*), shell gland (*s. g.*), and uterus (*ut.*). Enlarged. Original.

FIG. 135.—Transverse section, shows oral sucker (*o. s.*), perisuctorial space (*p. s. sp.*), and follicles of right vitellogene gland (*v. g.*). Enlarged. Original.

FIG. 136.—Transverse section through caudal extremity of right (caudal) testis (*t.*). Shows position and relations of ovary (*ov.*), shell gland (*s. g.*), vitelline reservoir (*v. r.*), uterus (*ut.*), vitellaria (*v. g.*), intestinal ceca (*i.*), and acetabulum (*ac.*). Enlarged. Original.

STEPHANOPHARYNGINÆ, new subfamily.

SUBFAMILY DIAGNOSIS. (*Paramphistomidæ*, p. 60): Oral sucker with circular evagination.

TYPE GENUS.—*Stephanopharynx* Fischœder, 1901.

The circular evagination of the oral sucker appears to be an important character. At first thought it seems more important than the sexual characters, still, when one recalls that the intestinal characters of the distomes are not of such great value as one is tempted to accord to them, it is not absolutely excluded that subfamily value is higher than this character justifies. Accordingly, this subfamily is proposed with some reserve.

The one genus (*Stephanopharynx*) known for this group, presents also the following important characters: Genital sucker and cirrus pouch absent, body not divided.

Fischœder classified *Stephanopharynx* in *Paramphistominæ*. With this view it is difficult to concur, and if *Stephanopharynx* were classified with the *Cladorchiinæ*, there should be a distinct tribe erected for it.

Genus STEPHANOPHARYNX Fischœder, 1901.

GENERIC DIAGNOSIS.[a] *Stephanophayrngina* (p. 168): Body compressed, slightly concave ventrad, convex dorsad, but slightly flattened dorso-ventrally, cephalic and caudal ends rounded, sides excurvate longitudinally. Ventral pouch absent. Acetabulum ventro-subterminal, large, not sunken, margin not raised, aperture large. Genital pore with considerable musculature which is not sharply defined in form of a sucker, atrium divided into large ventral and small dorsal chamber, ductus hermaphoditicus present. Excretory pore prevesicular in acetabular zone, caudad of Laurer's anal. Oral sucker with circular evagination, larger dorsally than ventrally; esophgus without muscular thickening; ceca very wavy, end postequatorial, posttesticular.

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[a] Based on Fischœder, 1903h.

Male organs: Testes 2, smaller than acetabulum, with small lobes, fields nearly oincide, zones abut or separate, preovarial, not widely separated from acetabulum, equatorial and caudal thirds; musculosa well developed, but not enormous; cirrus ouch absent.

Female organs: Ovary and shell gland posttesticular; vitellaria pre- and cecal, rofuse; uterus intercecal; eggs many; Laurer's canal entirely preexcretory.

TYPE SPECIES.—*S. compactus* Fischœder, 1901.

Subfamily CLADORCHIINÆ Fischœder, 1901.

SUBFAMILY DIAGNOSIS.—*Paramphistomidæ* (p. 60): Oral sucker with a pair of vaginations.

TYPE GENUS.—*Cladorchis* Fischœder, 1901.

Fischœder included in this subfamily the genera *Cladorchis* (subg. *Cladorchis*, *Taxorchis*, and *Stichorchis*), *Chiorchis*, *Gastrodiscus*, *Homaogaster*, and (as doubtful) *Diplodiscus*.

We have separated out (see p. 249) *Gastrodiscus* and *Homalogaster*, thereby restricting the group in one sense, and by eliminating certain characters from the subfamily diagnosis we have widened the scope of the group in another sense. It is very possible that the group may undergo further changes on basis of the different kinds of evaginations.

Genus CLADORCHIS Fischœder, 1901.

GENERIC DIAGNOSIS.[a]—*Cladorchiinæ* (p. 169): Genital pore with sucker. Evaginations of oral sucker recognizable outside the wall of the sucker; esophagus without muscular thickening.

Male organs: Testes branched.

TYPE SPECIES.—*C. pyriformis* (Diesing, 1838) Fischœder, 1901.

Fischœder divides this genus into 3 subgenera, but it seems to us possible that his genus *Chiorchis* is perhaps of tribal value and his subgenera of generic value.

Subgenus CLADORCHIS Fischœder, 1901.

SUBGENERIC DIAGNOSIS.[a]—*Cladorchis* (p. 169): Body rather pyriform, venter flattened to convex, dorsum convex, cephalic end attenuate, caudal end rounded. Ventral pouch absent. Acetabulum caudal, ventral, rather large to large, apparently sunken or not sunken, margin apparently raised or not raised, aperture circular, apparently medium to large. Genital pore with sucker, ductus hermaphroditicus present. Excretory pore postvesicular, post- or acetabular, caudad of pore of Laurer's canal. Oral sucker with paired strongly developed evaginations, and with not sharply bounded sphincter; esophagus without muscular thickening; ceca very wavy, long, end postequatorial, posttesticular, post- or acetabular.

Male organs: Testes 2, smaller than acetabulum, branched, fields abut, zones nearly coincide, preovarial, near acetabulum, in equatorial third, near venter; cirrus pouch present.

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[a] Based on Fischœder, 1903h.

Female organs: Ovary and shell gland posttesticular; vitellaria in cecal zone, testicular and post-, may be pretesticular, stretching ventrally and dorsally; uterus intercecal; Laurer's canal entirely prevesicular.

TYPE.—*C. pyriformis* (Diesing, 1838).

HABITAT.—Cecum of South American *Tapirus*.

Subgenus STICHORCHIS Fischœder, 1901.

GENERIC DIAGNOSIS.[a]—*Cladorchis* (p. 169): Body straight, venter rather flat, dorsum convex, cephalic third notably attenuate, caudal end attenuate but rounded. Ventral pouch absent. Acetabulum caudal, ventral, slightly sunken, margin (?), aperture circular, large. Genital pore with sucker, latter with distinct sphincter. Excretory pore apparently postvesicular, in acetabular zone, caudad of pore of Laurer's canal. Oral sucker with sphincter and paired evaginations; esophagus without muscular thickening; ceca slightly wavy, long, end postequatorial, posttesticular, in acetabular zone.

Male organs: Testes 2, about as large as acetabulum, branched, fields and zones overlap, preovarial, somewhat separated from acetabulum, near venter, in equatorial third; cirrus pouch small.

Female glands: Ovary and shell gland almost entirely posttesticular; vitellaria from bifurcal to postcecal zones; uterus intercecal; eggs (?); Laurer's canal entirely prevesicular.

TYPE SPECIES.—*S. giganteus* (Diesing, 1835).

Genus TAXORCHIS (Fischœder, 1901).

GENERIC DIAGNOSIS.[a]—*Cladorchiina* (p. 169): Body elongate, flattened, venter flat, dorsum somewhat convex. Ventral pouch absent. Acetabulum terminal, rather large, aperture elongate. Genital pore with sucker, ductus hermaphroditicus present. Excretory pore (?). Oral sucker with sphincter and well-developed paired evaginations; esophagus without muscular thickening; ceca broad, slightly wavy, near dorsum, long, end postequatorial, posttesticular, in acetabular zone.

Male organs: Testes 2, nearly as large as acetabulum, branched, fields separate, zones coincide, preovarial, preequatorial, widely separated from acetabulum and ovary; cirrus pouch present.

Female organs: Ovary and shell gland posttesticular, near acetabulum; vitellaria in cecal zone, entirely posttesticular; uterus intercecal, chiefly posttesticular, unusually well developed, first runs near dorsum cephalad to testes, then diagonally caudad near venter, then near venter cephalad to pore; ova numerous. Laurer's canal (?).

TYPE.—*T. schistocotyle* Fischœder, 1901.

HABITAT.—Cecum of Brazilian *Dicotyles*.

Fischœder gives *Taxorchis* as a subgenus of *Cladorchis*, but the form and position of the testes and the position of the uterus seem to us to entitle it to generic rank.

Genus PSEUDODISCUS Sonsino, 1895.

1895: *Pseudodiscus* and *Amphist.* (*Pseudodiscus*) Sonsino, 1895, 5, 8 (for *hawkesi*, *collinsi*, *ornatum*), 1895, 184, 185, 186; 1896, 310.—Fischœder, 1903h, 489, 631–632.—Piana & Stazzi, 1900a, 523.

GENERIC DIAGNOSIS.—*Cladorchiina* (p. 169): Body oval, venter convex to concave, dorsum convex, cephalic end less blunt than bluntly rounded caudal end, transverse section elliptical. Ventral pouch absent. Acetabulum ventral relatively small, margins prominently projecting. Genital pore postbifurcal without sucker, ductus hermaphroditicus present. Excretory pore postvesicular, in postacetabular zone, caudad of pore of Laurer's canal. Oral sucker prominently constricted at equator, with a pair of evaginated horns, each with a globular pouch; esophagus without muscular thickening; ceca wavy, long, end postequatorial, posttesticular, in acetabular zone.

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[a] Based on Fischœder, 1903h.

Male organs: Testes 2, smaller than acetabulum, cauliflower-like, testicular fields separate, zones coincide, preovarial, considerably or slightly removed from acetabulum, chiefly or entirely in equatorial third, near venter; musculosa not enormously developed; cirrus pouch absent.

Female organs: Ovary and shell gland chiefly posttesticular; vitellaria extend extracecal about from buccal pouches to acetabulum; uterus intercecal, chiefly posttesticular; ? eggs; Laurer's canal cephalad and dorsal of excretory vesicle.

Eggs: Not observed.

Type species.—*Amphist. stanleyii* Cobbold, 1875, from *Equus caballus* in India, type by present designation.

Hosts.—Horses and elephants.

This genus is left provisionally in the subfamily *Cladorchiinæ*, although indications are not entirely absent that it may eventually be eliminated from this group.

Sonsino (1895, anno 6, 5, 8) proposed this genus as a member of the *Amphistomidæ* and gave to it the following generic diagnosis:

> Corpo allungato, convesso pianeggiante, sensa manico anteriore distinto. Ventosa posteriore subterminale piccola.

He included in the genus the species: *Amphist. hawkesi* [*stanleyii*] *collinsi*, and *ornatum*.

Piana & Stazzi (1900, 523) accept Sonsino's genus, adding to its diagnosis the phrase: "La faringe coi due diverticoli e il bulbo esofageo musculosa."

Fischœder (1902a, 48–49) gives the species *Amphist. hawkesi*, *collinsi*, *stanleyi*, and *ornatum* as species inquirendæ.

Fischœder (1903h, 489, 631–632) mentions the genus *Pseudodiscus*, but in view of the slight anatomical details then known for its species he lists them all as species inquirendæ of *Amphistomum*.

In reference to the species which come into consideration as members of this genus, Cobbold (1879b, 357–359, 398) states:

> More importance attaches itself to the study of the amphistomatoid flukes [in equines]. These parasites, though in a scientific sense only recently discovered in equine bearers, have been long known to the natives of India. They appear to be capable of producing serious intestinal irritation. I have described two forms (*Amphistoma collinsii* and *A. coll.* var. *stanleyi*, which infests the colon. The specimens sent to Professor Simonds from India by Mr. Stanley, V. S., were much larger than those sent to me from Simla by Mr. Collins, V. S., some ten years later (1875). As in all other amphistomes obtained from the intestines of elephants and cattle the worms, when fresh, were of a bright brick-red color. By the natives of India these parasites are called *Masuri;* but no description of the worms had been published prior to the account which I gave of the contributions forwarded by Major-General Hawkes, Mr. Collins, and Mr. Stanley.
>
> I shall have occasion to speak of the elephant's *Masuri* further on; but in the meantime I must remark that the generally received notion as to the parasitic cause of the

earth-eating propensities of various animals seems to have some foundation in fact. Not alone from Major-General Hawkes in Madras, from Mr. Folkard in Ceylon, and from various other trustworthy sources, have I been informed of this habit on the part of Indian horses; but Doctor Rowe told me that Australian horses, and even sheep, infested with stomach worms, are in the constant habit of consuming large quantities of sand. From all the facts that have come before me, I am inclined to think that gastric or intestinal irritation, however brought about, may induce the habit in question, parasites being only one of the many sources of irritation giving rise to symptoms of colic in solipeds and pachyderms alike. At all events the African elephants at the London Zoological Society's menagerie, as repeatedly witnessed by myself, are in the habit of swallowing large quantities of soft mud during the summer months, but no traces of *masuri* have as yet been detected in their fæces.

When by letter I informed Major-General Hawkes of an interesting find by Mr. Collins of *about a thousand* Amphistomes in the colon of a horse that had died at Simla, the announcement called forth a reply which is sufficiently instructive to be quoted. Writing from Secunderabad, in July, 1875, he says respecting this "find:" "Your statement has incidentally thrown light upon a subject which has puzzled many of us in this country. It occasionally happens that a horse, on being opened after death, is found to have accumulated in his intestines large quantities of sand and gravel. In a recent case this accumulation amounted to 14½ pounds. Until recently it was always held that this gravel or sand could only be introduced with the animal's food. All grain in this country is trodden out by bullocks on an earthen floor, and the grain undoubtedly contains a proportion of sand and gravel derived from this source. Although this ought to be carefully washed out before it is given to the horse, still, owing to the carelessness of the native horse keepers, this cleaning is, I expect, often omitted. In the daily 'feed' of 8 or 10 pounds of grain given to each horse the utmost quantity of sand or gravel that could be found admixed therewith would not probably exceed 2 or 3 ounces. Consequently it would take from seventy-seven to one hundred and sixteen days to accumulate so large a quantity as 14½ pounds. Now, the advocates of the theory of the gradual accumulation of sand in this way have never been able to explain why the grain, grass, hay, and other ingesta should pass in the ordinary way through the intestines whilst this sand or gravel remains behind. One can understand the possibility of such substances as wool, hair, or similar matters concreting in the alimentary canal, though I believe they are usually found in the stomach and not in the intestines; but how a most incohesive substance, like sand, can possibly accumulate in the gradual way required by their theory I have never heard even plausibly explained. On the other hand, the fact that horses are often excessively addicted to eating earth is well known; and if my memory serves me correctly, it was found necessary, about twenty years ago, to remove the mud walls of the pickets surrounding some of the horses of a mounted corps in this presidency in consequence of this habit. Now, given the fact that the amphistoma has been found in the horse (as your specimens prove), may we not fairly suppose it possible that the animal resorts to the same mode of ridding himself of this parasite as does the elephant; and, also, would it not in a much more natural manner account for the large quantity of gravel or sand found in the intestines than does the theory of gradual accumulation? Reasoning from analogy, as in the case of the elephant, this eating of earth in the horse would be an instinctive effort on the part of the 'host' to rid himself of the parasite. This self-taken remedy is doubtless in many cases quite effectual, though unnoticed. The fatal cases are probably those in which the horse has either overdone the remedy or where the system was too debilitated to carry off a quantity of sand or gravel that would otherwise have safely passed through the intestines of a horse in more robust health. The actual fact must, of course, be verified by careful investigation."

When describing the parasites of the horse (p. 358), I spoke of Collins's amphistome from that animal; but in the letter addressed to me from Simla, March 22, 1875, Mr. Collins made no allusion to the earth-eating habit. He wrote: "I forward you by this mail parasites found in the colon of a horse that died, a subject of fever peculiar to this country. There were about a thousand of the parasites, and nearly the whole of them were situated close to the cæcum and were loose in the gut. Not having seen parasites at all similar to these, I have forwarded them for identification. They were of a brick-red color when first obtained." These explicit statements by Mr. Collins are interesting from many points of view. One has only to place his specimens side by side with those from the elephant in order to satisfy one's self that the two forms are distinct. For the reasons already stated, I provisionally called the worm *Amphistoma collinsii.* It is probable that other veterinary surgeons have encountered this entozoon in India; but unless they can point to some published account of the fact Mr. Collins is entitled to be considered as its discoverer. Doubtless many other European residents in India, Ceylon, and Burmah must, like Doctor Gilchrist, be well acquainted with the *masuri* as such, though unaware of their zoological position.

The known species[a] of *Pseudodiscus* may be distinguished by the following key:

a^1. Testicular zones nearly or quite coincide, fields separate; esophagus without muscular thickening..............................subg. *Pseudodiscus*, p. 173.
  b^1. Testes more than half as large as acetabulum; intertesticular field less than half as broad as testicular field; body 8.6 to 9 mm. long; type host *Equus caballus*, India..................................*Ps. stanleyii*, p. 173.
  b^2. Testes less than half as large as acetabulum; intertesticular field much broader than testicular field; body 5 to 5.7 mm. long; type host *Equus caballus*, India..*Ps. collinsii*, p. 187.
a^2. Testicular zones separate, nearly abut, fields nearly or quite coincide; esophageal muscular thickening present; tpye *hawkesii*...........subg. *Hawkesius*, p. 200.
  b^3. Body 3.5 to 5 mm. long; type host *Elephas indicus*, India...*Ps. hawkesii*, p. 200.

Subgenus PSEUDODISCUS.

Subgeneric diagnosis.—*Pseudodiscus* (p. 170): Esophageal muscular thickening absent. Testicular zones nearly or quite coincide, fields separate.

Type species.—*Ps. stanleyii.*

PSEUDODISCUS STANLEYII (Cobbold, 1875) Stiles & Goldberger, 1910.

[Figs. 137 to 151.]

1875: *Amphist. stanleyii* Cobbold, 1875n, 818, 819 as possible syn. of *Amphist. collinsii* [in *Equus caballus*, India].

1879: *Amphist. collinsii* var. *stanleyi* Cobbold, 1879b, 357 for *stanleyii* 1875 (in *Equus*).—Piana & Stazzi, 1900a, 519, as syn. of *hawkesi*.—Sons., 1895, 182.

1895: *Amphist. collinsi* var. *stanleyi* Cobbold.—Sons., 1895, 4 syn. of *Amphist. hawkesi*.—Fischœder, 1902a, 48; 1903h, 631, 632.

1895: *Amphist. stanleyi* Cobbold.—Ward, 1895, 338 as syn. of *A. collinsi* (in *Equus caballus*).—Fischœder, 1902, 48.

Specific diagnosis.—*Pseudodiscus* (p. 170): Body 8.6 to 9 mm. long by 5.5 to 5.6 mm. broad, by 3.5 to 4 mm. thick; flesh color (alcohol specimen); oval, somewhat flattened dorso-ventrally, cephalic extremity bluntly pointed, caudal extremity very bluntly rounded; lateral margins convex, greatest diameter (both transverse and sagittal) about at caudal end of equatorial third of body; cephalic extremity bears bluntly pointed slender papillæ. Genital pore ventro-median in cephalic portion of equatorial third, about midway between oral extremity and anterior margin of acetabulum, postbifurcal. Acetabulum ventro-subterminal, 1.7 mm. in diameter, aperture circular, 1 to 1.25 mm. in diameter, surrounded by a distinctly raised margin; cavity rather shallow. Mouth terminal; oral sucker constricted at equator into globular oral and bibulbous esophageal portions; each lateral bulb connects with a globular pouch; the bibulbous portion of sucker and the pouches lie on each side in a cavity, but are bound dorsally and ventrally to the parenchyma by mesenterium-like bands; esophagus arises from base between bulbs and extends (at first in the ventral mesenterium-like band) nearly to border between cephalic and equatorial thirds of body; ceca long, extend caudad slightly beyond equator of acetabulum, each forming in its course 2 strongly convex lines latero-dorsad, which come together near caudal margin of testis. Excretory pore dorso-median, caudad of acetabulum; excretory vesicle well developed, dorsal of acetabulum.

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[a] Except *ornatus*, for which definite data are lacking.

Male organs: Testes large, somewhat lobulated or cauliflower-like, ventral, equatorial, postbifurcal, preacetabular, their center slightly nearer caudal than cephalic margin; vasa efferentia spring from dorsal aspect, run cephalo-mediad, then mediad, unite about in median line slightly caudad of equator of body to form vas deferens; vesicula seminalis intricately coiled, moderately dilated; pars musculosa dilated, moderately coiled, extends slightly cephalad of genital pore; pars prostatica relatively short, vesicular, dorsal of pore, with short narrow muscular canal (ductus ejaculatorius) to the short ductus hermaphroditicus which leads to the pore; cirrus pouch absent.

Female organs: Ovary and shell gland submedian, posttesticular, preacetabular, nearer acetabulum than testes, ovary cephalad of shell gland; vitellaria, with well-developed follicles, lateral of ceca, extend about from base of esophageal pouches to or slightly caudad of intestinal ceca; vitello-ducts arise about at plane of shell gland; uterus forms coils dorso-mediad of ovary, then passes cephalo-ventrad in suctorial field between testes, crossing ventrally of vasa efferentia, and runs in but slight coils ventrad of vas deferens to enter ductus hermaphroditicus dorsal of pore; Laurer's canal runs from its origin (between dorsal margin of ovary and shell gland) caudo-dorsad to dorso-median line slightly caudad of cephalic margin of acetabulum.

Eggs: Not observed.

TYPE.—U.S.N.M. (Coll. Stiles) 5274; cotype U.S.N.M. (Coll. Hassall) 5779, sectioned and used as basis for present anatomical discussion.

HABITAT.—Colon of the horse (*Equus caballus*), India.

SOURCE OF MATERIAL.—The material used as basis of this discussion represents some of Cobbold's original specimens which he gave to Hassall; the latter brought them to this country. Cobbold was not in the habit of designating any particular specimen as type, but Hassall has designated 5274 as such on the label.

HISTORICAL REVIEW.—In the first mention of the name *Amphistoma stanleyii* Cobbold (1875n, 818, 819) states that the parasites were collected by Edward Stanley, jr., from the colon of the horse in India; in size they appear nearer to *A. hawkesii* than they do to *A. collinsii*, still "this is apparently nothing more than a large variety of the above [*collinsii*] (?)."

Ward (1895, 338) merely mentions this form as a synonym of *Amphist. collinsi*, in a list of parasites of the horse.

Sonsino (1895, 4), quoting Cobbold, mentions the worm as a synonym of *Amphist. hawkesi.*

Fischœder (1902a, 48; 1903h, 631, 632) quotes the measurements as 10 mm. long by 6 mm. broad, states that this form occurs in the colon of *Equus caballus* in India, and marks the parasite as a species inquirenda.

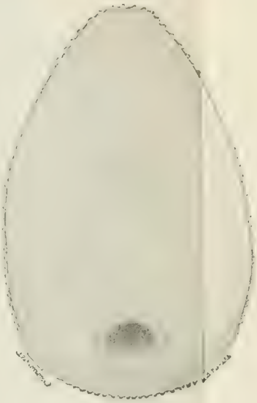

FIG. 137.

EXTERNAL CHARACTERS.

SIZE.—The specimens, preserved in alcohol, measure 9 mm. and 8.6 mm., respectively, in length; 5.5 and 5.6 mm. in greatest width, and 3.5 and 4 mm., respectively, in greatest dorso-ventral diameter.

COLOR.—The worms are of a flesh tint.

FORM.—The worms are ventro-dorsally somewhat flattened oval objects, with a bluntly pointed oral and a broad rounded caudal extremity (figs. 137, 138). The greatest transverse and ventro-dorsal diameters are about in the region of junction of the middle with the caudal third of the body. In transverse sections the form of the body is somewhat that of an ellipse.

SURFACE.—The surface of the oral pole is beset by small, more or less bluntly pointed, quite slender papillæ. They presented much the appearance of spines, but any doubt as to interpretation was dissipated on studying the sectioned specimen.

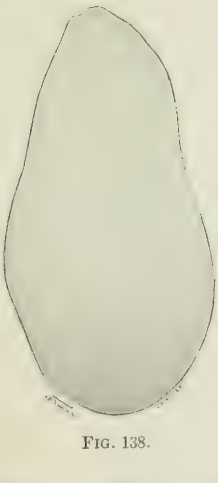

FIG. 138.

Genital pore.—On the ventral surface in the median longitudinal line, about midway between the oral extremity and the anterior margin of the acetabular aperture, is the genital pore. This pore is situated in about the center of a broad shallow depression of the ventral surface. In sections it is seen that what appears to be the genital pore is a fairly large circular aperture that leads into a circular slit-like atrium, on the dorsal wall of which appears a minute pore which leads into another, somewhat better defined, chamber. On the dorsal wall of the latter is the opening of the ductus hermaphroditicus. These atria and the ductus hermaphroditicus are surrounded by a globular mesh of muscular fibers (fig. 146).

Acetabulum.—The acetabulum is in the caudal portion of the body, with its aperture ventro-subterminal. In one specimen it measured (in sections) about 1.7 mm. in diameter; the aperture of the acetab-

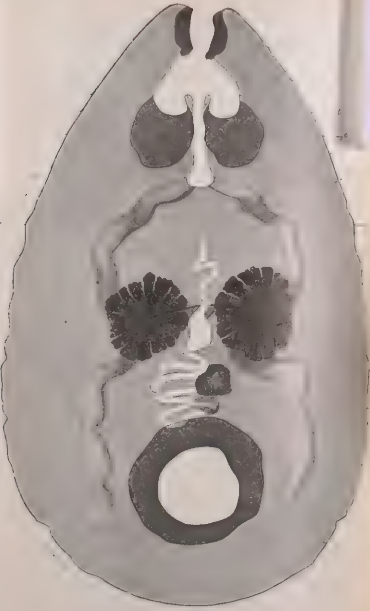

Fig. 59

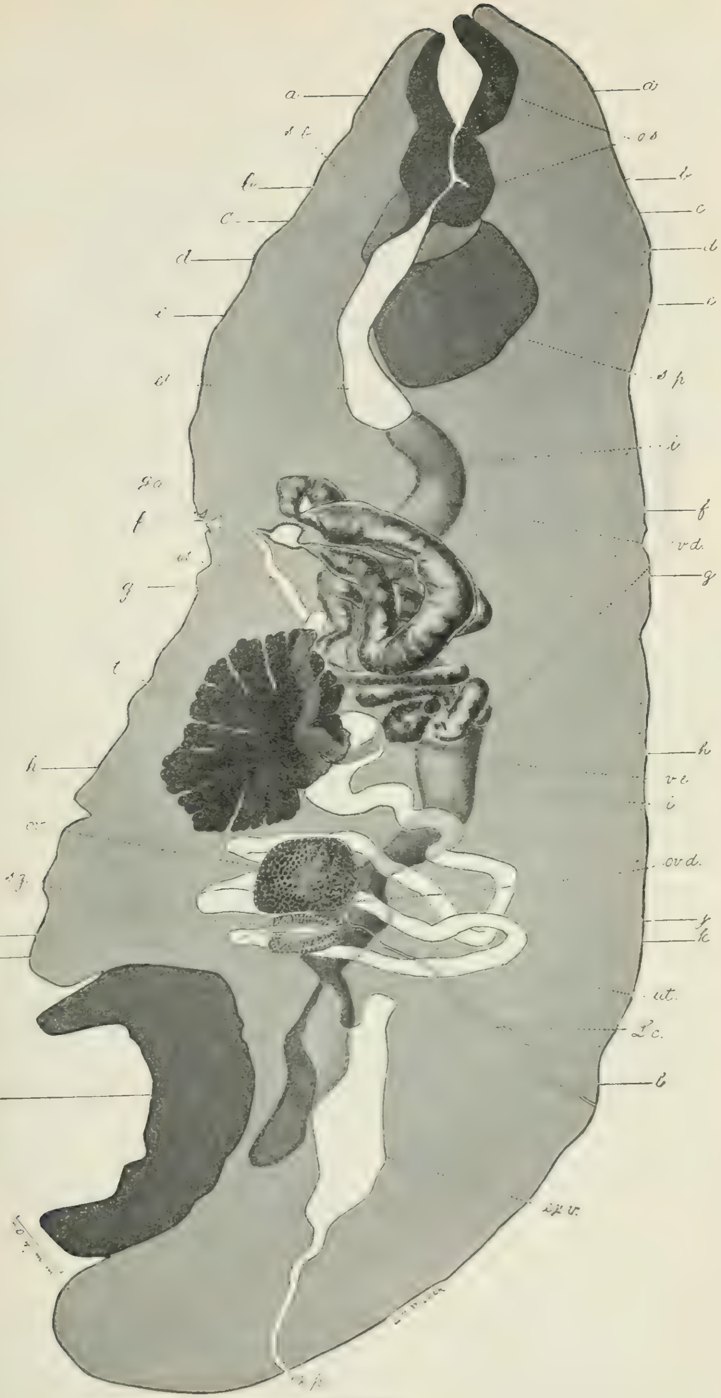
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FIG. 140.

ulum in the 2 worms measured 1 mm. and 1.25 mm., respectively, in diameter. This aperture is surrounded by a narrow ring which is marked off from the general surface by a deep and more or less narrow circular groove. This ring is seen in sections to be part of the acetabulum (fig. 151). The cavity of the acetabulum is relatively shallow.

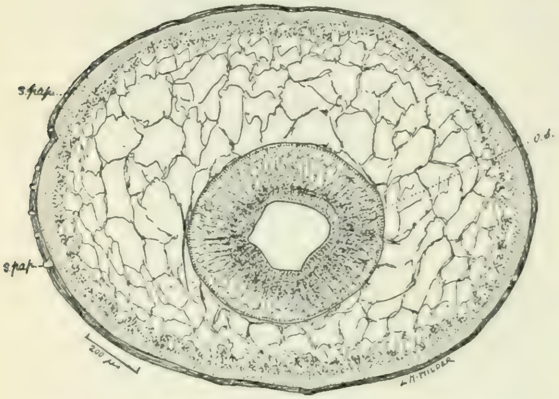

FIG. 141.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The oral aperture, which pierces the cephalic extremity of the worm and is unprovided with a sphincter, leads directly into a muscular sucker. The latter shows a well-marked constriction or isthmus at about its equator, which divides it transversely into two portions almost equal in length—the anterior, which may be designated as the oral, and the posterior, as the esophageal (or bulbous) portion. The oral portion of the sucker is somewhat globular in form, measuring (in sections) about 0.64 mm. in longitudinal axis, 0.64 mm. in transverse, and 0.58 mm. in ventro-dorsal diameter. The esophageal or bulbous portion differs in form quite markedly from the oral portion (figs. 139, 140, 141, 142, 143). It measures (in sections) about 0.50 mm. in longitudinal axis and 0.48 mm. in greatest ventro-dorsal diameter. In transverse diameter this part of the sucker is narrowest at the constriction marking its anterior limit, where it measures 0.40 mm., but it increases rapidly to a maximum width of 1.10 mm. at its base, namely, at the level at which the esophagus takes its departure. In sections it is seen that this increase in width is due to the formation of lateral bulbs which extend caudad on either side of and in line with the esophagus for a distance (measured in the long axis of the bulb) of about 0.40 mm. beyond

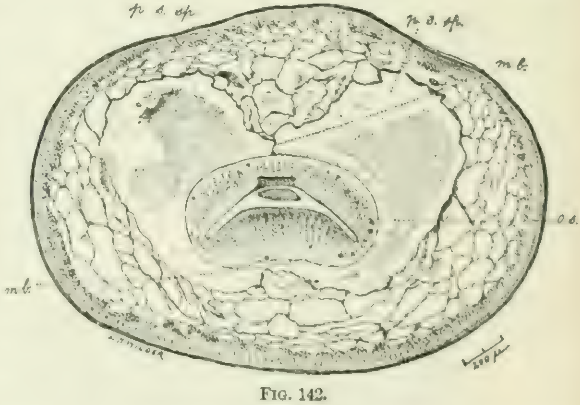

FIG. 142.

the level or plane of origin of the esophagus (figs. 139, 140). Attached to the dorso-caudal aspect of these bulbs there is on each side a globular pouch.

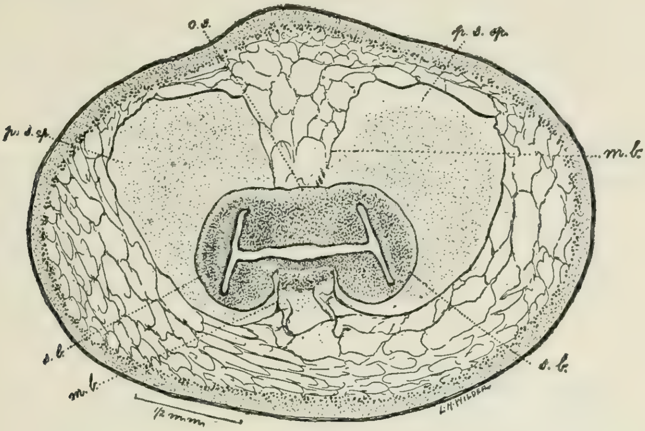

FIG. 143.

In consequence of this relation between the pouches and the corresponding bulbs, the former are found close to, on either side, and slightly dorsad of the esophagus. The suctorial bulbs, which are an integral part of the sucker, are, like the latter, muscular in structure. The portion of these bulbs which on surface inspection is seen to

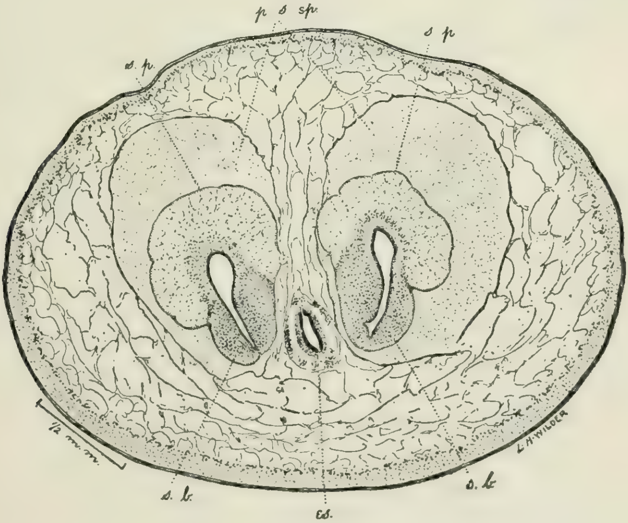

FIG. 144.

extend beyond the base of the sucker, that is, caudad of the level of origin of the esophagus, measures about 0.40 mm. in its longitudinal axis, about 0.56 mm. in its greatest dorso-ventral diameter, and about

0.40 mm. in its greatest transverse diameter. The suctorial "pouches" (which are here distinguished from the "bulbs") are in a general way globular in form. Their long axes have about the same direction as the long axis of the worm itself and exceed their transverse diameter by about 0.20 mm., the latter measuring about 0.80 mm. In structure the pouches are entirely different from the bulbs. The walls of each pouch, which measure about 0.30 mm., are made up of parenchyma-like cells and some muscular fibers. These parenchyma-like cells, though large, are much smaller than those of the body parenchyma, from which they are sharply separated by a thin membranous layer which at the same time forms the outer covering of the wall of the pouch. Beneath the lining cuticle is a layer of structures which are very irregular in size and which at first sight are not very easy to interpret; they have something of the appearance of poorly preserved nuclei; some seem to be solid, others appear to be hollow, with a peripheral row of dots; in some fields they remind the observer very strongly of cross-sections of longitudinal muscles, and it seems probable that they are such, for the terminal sections of the pouch show distinct fibers apparently corresponding to these structures. Immediately external to this layer is found a thin layer of circular muscles.

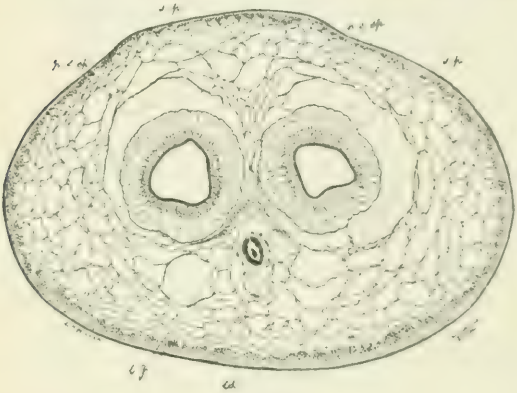

FIG. 145.

The portion of the sucker caudad of the isthmus, together with the two pouches, lies in a cavity which reminds the observer strongly of a rudimentary body cavity. From the dorsal and the ventral surfaces of the sucker a line of tissue, reminding the observer of a mesenterium, extends dorsad and ventrad to the body parenchyma, thus holding these structures in place; similar mesenterium-like bands connect the pouches with the somatic parenchyma (figs. 142-145); at their insertion, these bands spread around the sucker and pouches like a peritoneum; the esophagus runs in the ventral mesentery band, which also contains the esophageal ganglion.

Whether the cavities in question actually represent a rudimentary body cavity is a question which may be left open, but such an interpretation is rather tempting. Similar cavities are found or at least strongly indicated in *Homologaster philippinensis* (see Stiles and Goldberger, 1908a, figs. 34-36) *Watsonius watsoni*, and in other Paramphistomidæ. The lumen of the sucker differs in form in the different portions of the suctorial tract; that of the oral portion is somewhat spindle-shaped, while that of the esophageal portion is a transverse slit, slightly crescentic in both transverse and sagittal sections with the convexity directed dorsad. In about the equatorial plane of this portion of the sucker a shallow, transverse, slit-like diverticulum of the suctorial lumen is formed in the dorsal muscular wall, so that the lumen of the sucker in sagittal plane at this point presents a triradiate form (fig. 140); in transverse section at this point the impression obtained is that of a transverse tongue-like ridge or partition projecting upward into the lumen of the sucker (fig. 142). Caudad of this level the horns of the crescentic lumen seen in transverse section rapidly shift and assume a more or less direct dorso-ventral direction and form the dorso-ventral slit-like lumen of the bulbs so that in a section, say, at the level of origin of the esophagus, the lumen of the sucker and bulbs combined has a broad H-shape (fig. 143). The caudo-dorsal extremity of the slit-like lumen of the bulbs leads into the irregular lumen of the pouches (fig. 144). The sucker, bulbs, and pouches are lined throughout with a cuticle in anatomical continuation with that of the body surface. That of the second portion of the sucker and of its bulbs is provided with minute irregularly scattered conical papillæ. The esophagus (figs. 139, 140, 143, 145) springs from the base of the sucker in the space between the downward projections of the bulbs.

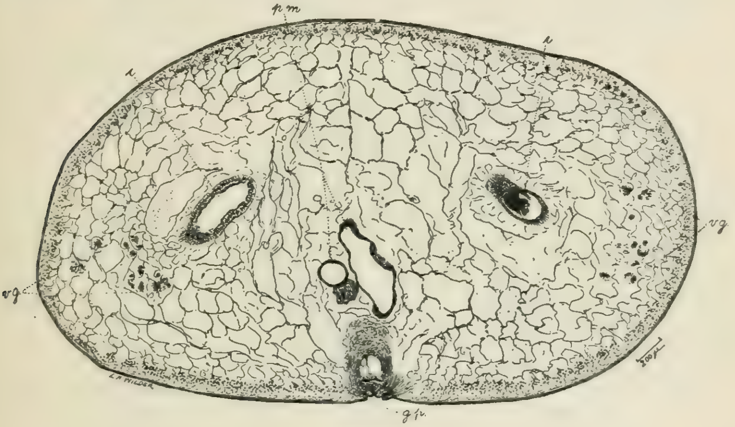

FIG. 146.

From this point it passes caudad, describing a slight curve ventrad, to a point but very little less than one-third of the body length from the oral extremity, slightly nearer the venter than the dorsum and about equidistant from the lateral margins, where it terminates, giving origin to the intestinal ceca. This terminal portion is somewhat expanded, and its musculature distinctly though not very greatly increased in thickness. The musculature of the esophagus consists of 2 layers, an internal of circular and an external of longitudinal fibers. The increase in thickness of the muscle wall of the caudal portion of the esophagus takes place gradually, and is due mainly to an increase in thickness of the internal circular layer. The intestinal tubes leave the latero-dorso-caudal aspect of the slightly-expanded caudal extremity of the esophagus and curve downward (caudad) and outward (laterad) and slightly dorsad, forming a transverse arch in the first part of their course. They terminate by blind extremities in the caudal portion of the body, the left at a very slightly higher level than the right, and both slightly caudad of a transverse plane through the equator of the acetabulum and about midway between the latero-dorsal aspect of the latter and the corresponding aspect of the body surface of the worm (figs. 139, 140, 151). In their course caudad the intestinal ceca describe an undulating path. The undulations are of moderate amplitude, directed from front (venter) to back (dorsum) and maintain about an equal distance from the latero-dorsal, curved surface of the worm. The ceca approach nearest the dorso-median longitudinal line in the testicular zone, and reach a position dorsad of the caudal portions of the corresponding testis. The diameter of the intestines varies somewhat in different portions, and although they are, in a general way, cylindrical tubes, yet they are compressed here and there in one diameter or another, so that in transverse sections they present quite a variable outline. The lumen of the esophagus is lined with a cuticular layer in anatomical continuation with that of the sucker, but apparently somewhat thicker than that of the latter. This lining ceases abruptly at the beginning of the intestinal ceca, which are lined throughout with an epitheial cell layer.

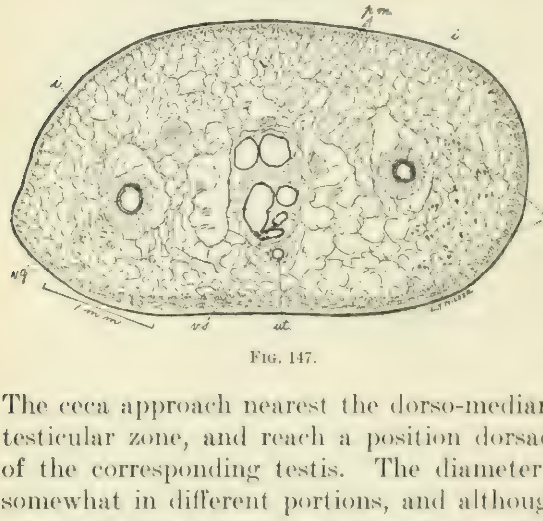

Fig. 147.

Genital system.—The male and the female genital organs, with the exception of the vitellogene glands, are disposed in the median field bounded laterally by the intestinal ceca.

Male organs.—This worm is provided with 2 fairly large lobulated (cauliflower-like) testes (fig. 148). They are placed one on each side of and close to the median sagittal plane in the same zone, slightly nearer the caudal than the oral extremity and decidedly nearer the venter than the dorsum. There is but slight if any difference in the size of the two organs. Such difference as may exist is in favor of the left.

From the dorsal aspect of each testis there emerges a vas efferens (fig. 148), which is directed at first slightly cephalo-mediad, then more directly mediad to unite with its fellow at a point in about the median sagittal plane, slightly nearer the caudal than the oral extremity, and considerably nearer the dorsum than the venter, to form the vas deferens. The first portion of the vas is an intricately coiled thin walled moderately-dilated duct or vesicula seminalis. At a point which may be clearly distinguished (fig. 147) the thin walled vesicula gives place to a relatively thick (15μ) muscular walled canal (pars musculosa) of considerable caliber (150μ), which is much less intricately coiled, so that its longitudinal windings may be followed in transverse sections. A valve-like constriction of the lumen marks the change from vesicula to pars musculosa. The windings of these two portions of the vas deferens form a fairly compact mass, which lies in the axial region of the body between and cephalo-dorsad of the testes, and longitudinally between the equatorial plane and a transverse plane slightly cephalad of the genital pore. The terminal portion of the vas deferens is relatively quite short, and, like that of the pars musculosa, its beginning is clearly and sharply marked by a change in the character of the wall, which becomes thin and surrounded by glandular cells, which are relatively few in number, and by a valve-like constriction of the lumen. This portion of the vas deferens, which by analogy may be called the pars prostatica, is in the form of a small vesicle dorsal of the genital pore. From its ventral aspect there passes ventrad a short muscular duct (ductus ejaculatorius), which unites with the metraterm to form a ductus her-

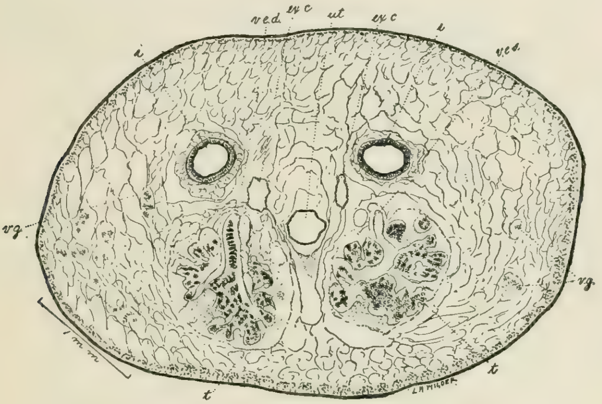

FIG. 148.

maphroditicus. The latter, a short delicate canal, opens into a small chamber, which in its turn opens into a slit-like genital atrium (fig. 140).

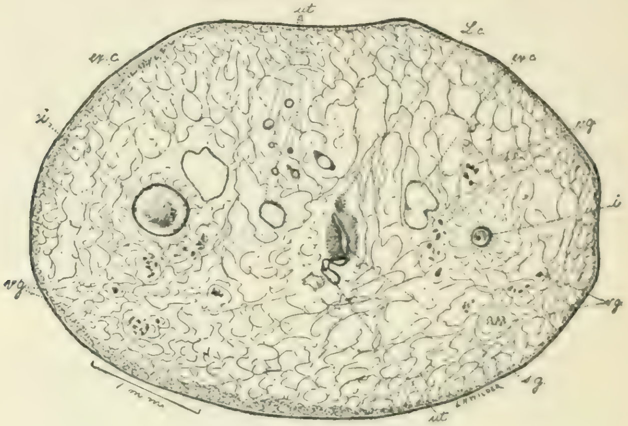

FIG. 149.

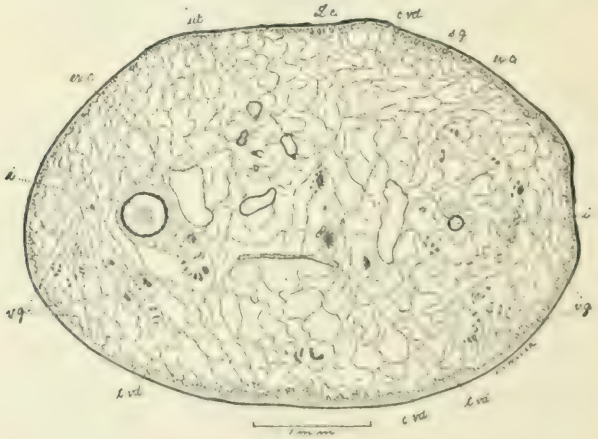

FIG. 150.

Female organs.—In the axial region of the body, a little to the left of the median line, between two transverse planes, one of which is just caudad of the caudal plane of the testes and the other just cephalad of the upper margin of the acetabulum, are the ovary and shell gland, the former just above (cephalad) and slightly to the left of the latter. From the dorso-caudal aspect of the ovary there springs the oviduct, which turns caudad as it passes to the dorsal aspect of the shell gland at the superior margin of which it divides into two branches, one of which curves slightly to the right to penetrate the dorsal aspect of the shell gland, in the substance of which it is joined by the vitello-duct to form the ootype. The shell gland is considerably smaller than the ovary, immediately caudad of which it lies. It is penetrated by the vitello-duct and, as just described, by the oviduct on its caudo-dorsal aspect. These two ducts unite to form a fusiform ootype (fig. 149), which is directed dorso-ventrally and is continued as the uterus, the latter emerging from the ventral aspect of the gland. The uterus, after emerging from the shell gland, curves to the right, then dorsad, and describes some coils in the axial region of the body to the right and dorsad of the shell gland and ovary, as it winds its way cephalad. At about the level of the superior aspect of the ovary it passes obliquely cephalo-ventrad to reach the ventral aspect of the coiled vas deferens. From this point onward its course is but slightly wavy, maintaining throughout its relation to the vas deferens as it passes at first directly cephalad, then gradually tilts ventrad, uniting with the ductus ejaculatorius at about the level and a short distance dorsad of the genital pore. The second of the two ducts into which the oviduct has been described as branching at the level of the superior margin of the shell gland is Laurer's canal (fig. 140). This canal passes caudo-dorsad to open in the middle line of the dorsal surface, a little caudad of the level of the upper margin of the acetabulum. The vitellogene glands, consisting of well-developed follicles, occupy the lateral regions of the body, external to the intestinal ceca, and extend from about the level of the plane of the base of the esophageal pouches to or slightly caudad of the plane of the cecal extremities of the intestines. At about the level of the shell gland a duct leaves each of the vitellogene glands and passes transversely inward ventrally of the ceca, the two uniting at the level of and close to the ventro-caudal margin of the shell gland (fig. 150). From their point of junction a duct passes dorsad, skirting the caudo-mesial margin of the shell gland and penetrating the latter at its caudo-dorsal aspect.

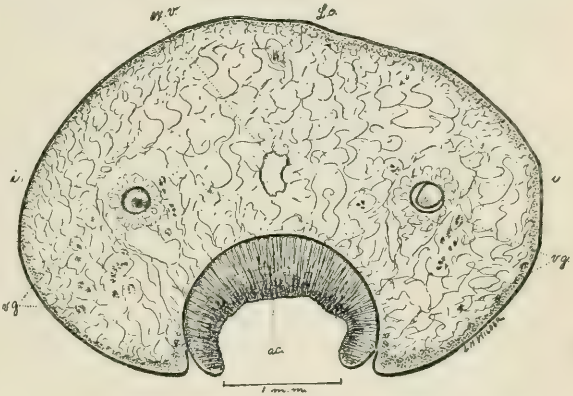

FIG. 151.

EXCRETORY SYSTEM.—This is highly developed. Two large and several small excretory canals enter the cephalic aspect of the excretory vesicle, which lies in the caudal portion of the axial region of the body dorsad of the acetabulum (figs. 140, 151). The vesicle is an elongate sack, the lumen of which as it extends caudad gradually becomes reduced in caliber, finally becoming a narrow canal, the terminal portion of which is lined with a cuticle in anatomical continuation with that of the surface. This terminal portion or excretory duct opens in the middle line of the dorsal surface only a little above the caudal margin of the worm and at a considerable distance caudad of the opening of Laurer's canal.

ILLUSTRATIONS.

Fig. 137.—Ventral view of *Ps. stanleyii*. Enlarged. Original.

Fig. 138.—Profile view of same. Enlarged. Original.

Fig. 139.—Frontal projection. Shows oral sucker (*o. s.*), suctorial bulbs (*s. b.*), suctorial pouches (*s. p.*), esophagus (*es.*), intestines (*i.*), testes (*t.*), vasa efferentia (*v. e.*), ovary (*ov.*), shell gland (*s. g.*), uterus (*ut.*), position of genital pore (*g. p.*), and acetabulum (*ac.*). *a–a, b–b, c–c, d–d, e–e, f–f, g–g, h–h, j–j, k–k*, planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 140.—Profile projection. Shows oral sucker (*o. s.*), suctorial bulb (*s. b.*), suctorial pouches (*s. p.*), esophagus (*es.*), intestines (*i.*), right testis (*t*), right vas efferens (*v. e.*), vas deferens (*v. d.*), ovary (*ov.*), shell gland (*s. g.*), uterus (*ut.*), oviduct (*ov. d.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), excretory pore (*ex. p.*), and genital atrium (*g. a.*). *a–a, b–b, c–c, d–d, e–e, f–f, g–g, h–h, j–j, k–k*, planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 141.—Transverse section at *a–a* figs. 139 and 140. Shows form of body, surface papillæ (*s. pap.*) and oral sucker (*o. s.*). Enlarged. Original.

Fig. 142.—Transverse section at *b–b* figs. 139 and 140. Shows form of body, form of esophageal portion of sucker (*o. s.*), perisuctorial space (*p. s. sp.*), and dorsal and ventral mesenterium-like bands (*m. b.*). Enlarged. Original.

Fig. 143.—Transverse section at beginning of esophagus through plane at *c–c* figs. 139 and 140. Shows form of body, form of oral sucker (*o. s.*), H-shaped form of suctorial lumen, perisuctorial space (*p. s. sp.*), and dorsal and ventral bands (*m. b.*). Enlarged. Original.

Fig. 144.—Transverse section through plane *d–d* figs. 139 and 140. Shows form of body, esophagus (*es.*), extension of lumen of suctorial bulb (*s. b.*) into suctorial pouch (*s. p.*), and caudal extension of perisuctorial space (*p. s. sp.*), which is filled with a granular coagulum. Enlarged. Original.

Fig. 145.—Transverse section through plane *e–e* figs. 139 and 140. Shows position and relations of suctorial pouches (*s. p.*) to esophagus (*es.*), the esophageal ganglion (*e. g.*), and the caudal extensions of the perisuctorial space (*p. s. sp.*), which contains some granular coagulum. Enlarged. Original.

Fig. 146.—Transverse section through plane at *f–f* figs. 139 and 140. Shows genital pore (*g. p.*), pars musculosa (*p. m.*), the intestinal ceca (*i.*), and the vitellaria (*v. g.*). Enlarged. Original.

Fig. 147.—Transverse section through plane *g–g* figs. 139 and 140. Shows position and relations of intestines (*i.*), pars musculosa (*p. m.*), the valve-like junction of the vesicula seminalis (*v. s.*) with the pars musculosa, the uterus (*ut.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 148.—Transverse section through plane *h–h* figs. 139 and 140. Shows position and relations of the testes (*t.*), the point of origin of the right (*v. e. d.*) and the left (*v. e. s.*) vas efferens, uterus (*ut.*), intestinal ceca (*i.*), vitellaria (*v. g.*), and excretory canals (*ex. c.*). Enlarged. Original.

Fig. 149.—Transverse section through plane *i–i* figs. 139 and 140. Shows position and relations of shell gland (*s. g.*), Laurer's canal (*L. c.*), uterus (*ut.*), intestines (*i.*), vitellaria (*v. g.*), and excretory canals (*ex. c.*). Enlarged. Original.

Fig. 150.—Transverse section through plane *k–k* figs. 139 and 140. Shows position and relations of transverse vitello-ducts (*t. vd.*), the common vitello-duct (*c. vd.*), base of shell gland (*s. g.*), Laurer's canal (*L. c.*), the uterus (*ut.*), vitellaria (*v. g.*), intestinal ceca (*i.*), and excretory canals (*ex. c.*). Enlarged. Original.

Fig. 151.—Transverse section at plane *l–l* figs. 139 and 140. Shows position and relations of Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), vitellaria (*v. g.*), intestines (*i.*), and acetabulum (*ac.*), with its projecting rim. Enlarged. Original.

PSEUDODISCUS COLLINSII (Cobbold, 1875) Stiles & Goldberger, 1910.

[Figs. 152 to 162.]

1875: *Amphist. collinsii* Cobbold, 1875l, 741 (in *Equus caballus;* Simla, India); 1875n, 818, 819; 1879b, 357, 359; 1883x, 515.—Fischœder., 1903h, 489 to *Pseudodiscus* by Sonsino.—Huber, 1896a, 580 (India).—Sonsino, 1895, 182, fig. 2; 1895, 4–5, fig. 2.—Theobold, 1900, 51.

1895: *Amphist.* (*Pseudodiscus*) *collinsi* (Cobbold, 1875) Sonsino, 1895, 182, 187; fig. 2; 1895, 9, fig. 2.

1895: *Amphist. collinsi* Ward, 1895, 338 (in *Equus caballus*).—Fischœder, 1902a, 48 (*E. c.*; India); 1903h, 631, 632.

Specific diagnosis.—*Pseudodiscus* (p. 170): Body 5 to 5.76 mm. long by 3.5 to 4 mm. broad; brick red (fresh) or flesh tint (alcohol specimens); oval, cephalic extremity somewhat blunted, caudal extremity very broadly rounded, nearly semicircular, lateral margins convex, greatest diameter slightly caudad of equator, about in testicular zone; cephalic extremity bears minute, slender, conical papillæ. Genital pore ventro-median about nine twenty-thirds of length from anterior end, in cephalic portion of equatorial third, postbifurcal, halfway between oral margin and cephalic margin of acetabulum. Acetabulum ventro-subterminal, 1.58 mm. broad, 1.1 mm. long, aperture 0.5 to 0.7 mm. in diameter, surrounded by very prominently raised margin; cavity relatively deep. Mouth terminal; oral sucker constricted at equator into a globular oral and a bibulbous esophageal portion; each lateral bulb connects with a large globular pouch; these pouches and a portion of the sucker lie in cavities strongly suggestive of a rudimentary body cavity, but they are connected with the body wall by dorso-ventral mesenterium-like bands; esophagus arises from base between bulbs and extends about to border between cephalic and equatorial thirds of body; *ceca* long, extend about to equator of acetabulum, each forming in its course two strongly convex lines latero-dorsad, which come together near caudal margin of testis. Excretory pore medio-terminal, caudal of acetabulum; excretory vesicle well developed, dorsal of acetabulum.

Male organs: Testes rather small, somewhat globular and cauliflower like, ventral, equatorial, slightly nearer median line than corresponding lateral margins; zones nearly coincide, fields separate; vasa efferentia spring from dorso-median aspect, run dorso-mediad, unite about in equator in median line to form vas deferens; vesicula seminalis and pars musculosa compactly coiled in median field, extending very slightly cephalad of genital pore; pars prostatica short; ductus ejaculatorius very short, opens above metraterm into an atrium; the latter appears to communicate with a slit-like atrium from which a slender ductus hermaphroditicus extends ventrad to open on vertex of the small genital papilla; cirrus pouch absent.

Female organs: Ovary and shell gland submedian, posttesticular, preacetabular, nearer acetabulum than testes, ovary cephalad of shell gland; vitellaria, with sparsely scattered follicles, lateral of ceca, extending from equatorial plane of esophagus to plane of caudal end of ceca; vitello-ducts pass ventrally of ceca; uterus forms coils dorso-medio-lateral of ovary, and from a point about on the cephalic plane of the ovary it passes ventro-cephalad in a somewhat wavy course, crossing ventrally of the vasa efferentia, and eventually the metraterm opens into a slit-like atrium into which the ductus ejaculatorius also discharges; Laurer's canal from its origin (dorsally of shell gland) runs dorsally of excretory vesicle caudo-dorsad to dorso-median line, over equator of acetabulum, its pore being some distance cephalad of excretory pore.

Eggs: Not observed.

TYPE.—U.S.N.M. (Coll. Hassall) 5778 (C in sections); cotypes U.S.N.M. (Coll. Stiles) 5266, and U.S.B.A.I. 1720; all from Cobbold's original material.

HOST.—The horse (*Equus caballus;* India).

SOURCE OF MATERIAL.—The material, consisting of six specimens in all, was obtained from bottles as follows: U.S.N.M. (Coll. Stiles) No. 5266, containing 1 specimen; U.S.N.M. (Coll. Hassall) No. 5778, containing 4 specimens; U.S.B.A.I. No. 1720, containing 1 specimen.

These specimens represent part of Cobbold's original material, presented by him to Hassall in 1882. The worms were collected from *Equus caballus* in India.

HISTORICAL REVIEW.—Cobbold (1875l, 741) quotes from a letter from Collins, dated Simla, as follows:

> I forward you by this mail parasites found in the colon of a horse that died a subject of fever peculiar to this country. There were about a thousand of the parasites, and nearly the whole of them were situated close to the cæcum, and were loose in the gut. Not having seen parasites at all similar to these, I have taken the liberty to forward them for identification.

Cobbold states that the worms are considerably smaller than are *Amphist. hawkesii* from the elephant, and he names the worm *A. collinsii.* In a later paper (1875n, 818, 819) he refers to them again very briefly and gives the colon as the habitat.

Ward (1895, 338) merely mentions the worm, with *Amphist. stanleyi* as synonym, in a list of the parasites of the horse.

Sonsino (1895, 4–5, fig. 2) gives the size as 7 mm. long by 5 mm. broad, figures the worm, and, referring to its similarity to *Amphist. hawkesii*, places it in his new genus *Pseudodiscus*.

Huber (1896a, 580) simply states that this species "which occurs in horses, is also said to give rise to fatal disease in India."

Fischœder (1901a, 48) merely cites this form as a species inquirenda, gives the measurements as 7 mm. long by 3 mm. broad, and states that it occurs in the colon of *Equus caballus* in India.

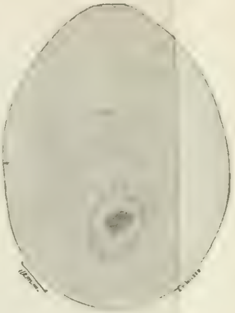

FIG. 152.

EXTERNAL CHARACTERS.

SIZE.—The 6 specimens preserved in alcohol-glycerine varied from 5 to 5.76 mm. in extreme length and from 3.5 to 4 mm. in greatest width.

COLOR.—The worms are of a flesh tint.

FORM.—The specimens are not in very good state of preservation and are contracted in various ways, so that all specimens are not uniform; on this account it is somewhat difficult to give an accurate description of the outline. In general, however, it may be said to be oval, with a somewhat blunted oral and a broadly rounded caudal extremity; it bears quite a resemblance to *Ps. stanleyii*, but the sides appear more uniformly convex, the cephalic end seems to have less tendency to be pointed, and the caudal extremity seems to be relatively more blunt (figs. 152, 153).

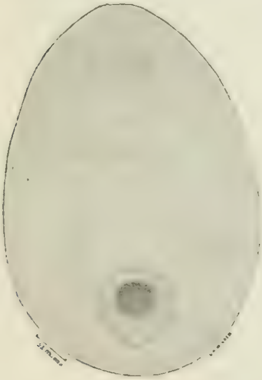

FIG. 153.

SURFACE.—At the oral pole the surface is beset by minute, slender, conical papillæ. They resemble those on *Ps. stanleyii* but are more minute. Around the oral aperture and concentric with it are a number of circular shallow grooves; the one nearest the oral margin appears rather prominent and marks off a narrow circular zone about the mouth. In some of the specimens there were noted fine transverse lines on the ventral surface. The dorsal surface is irregularly grooved, probably the result in part of irregular contraction of the body, and in part, perhaps, the result of the action of the fixative and preservative.

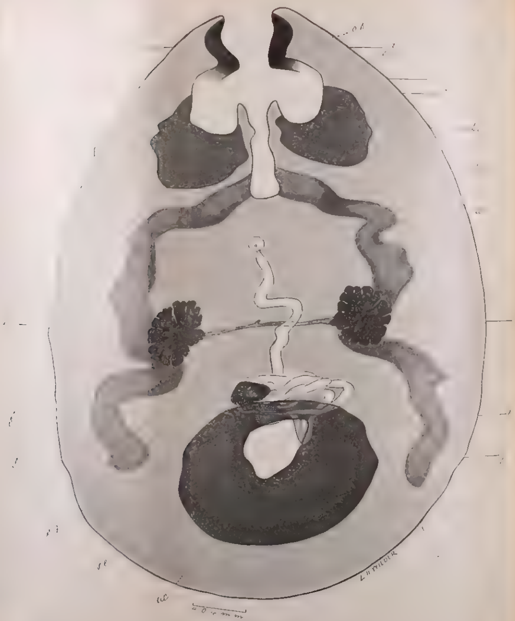

Fig. 14

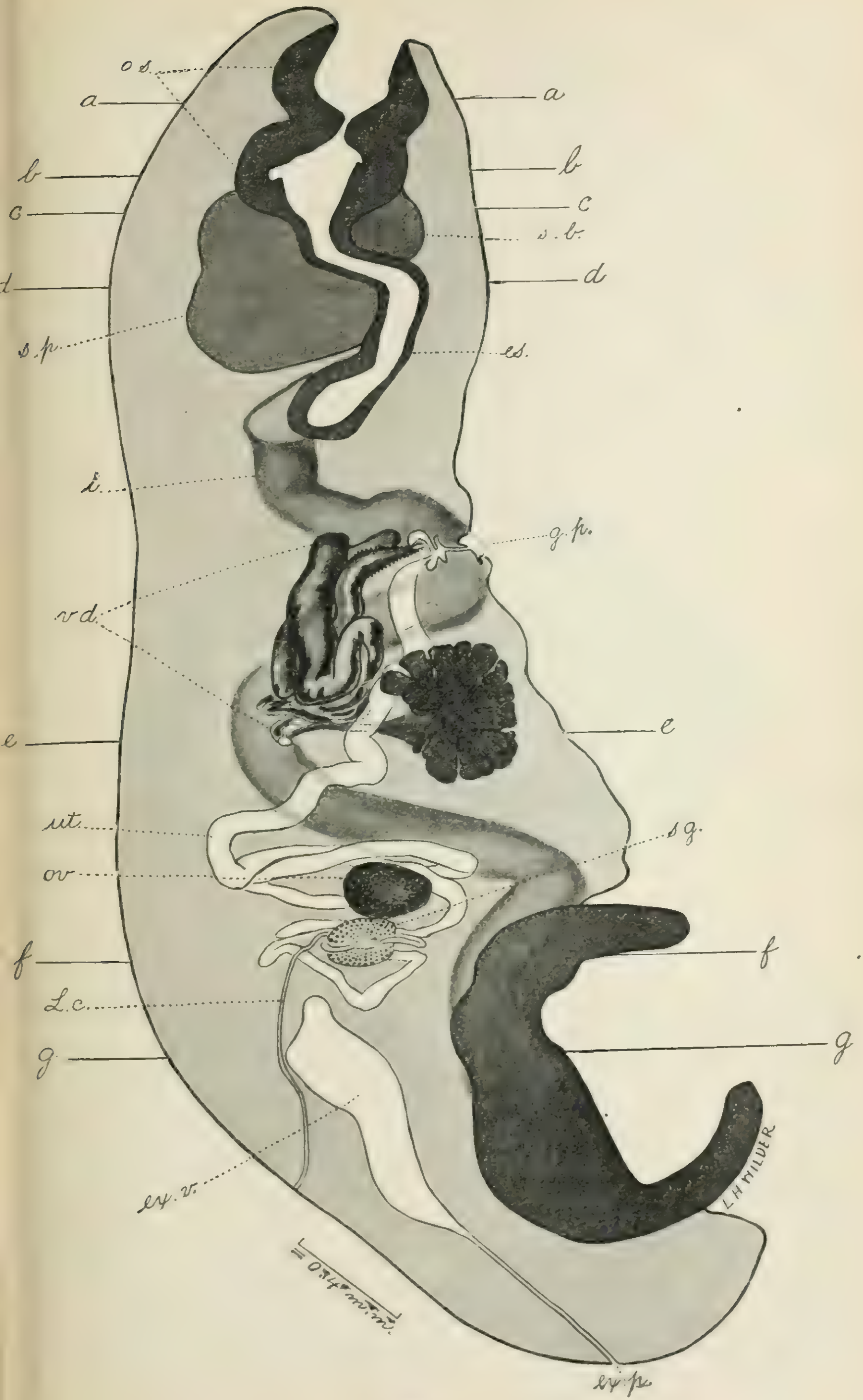
o.s.
a
a
b
b
c
c
s.b.
d
d
s.p.
es.
i.
g.p.
v.d.
e
e
ut.
sg.
ov
f
f
L.c.
g
g
ej.v.
= 0.4 mm.
ej.p.
L H WILDER

FIG. 155.

Genital pore.—On the ventral surface in the median longitudinal line and about midway between the oral margin and the anterior margin of the acetabular aperture is the genital pore. In one specimen this was at the vertex of a small circular bulging of the venter and, with the genital papillæ (apparently) filling it, suggested the form of an umbillicated smallpox vesicle.

Acetabulum.—The acetabulum, measuring 1.58 mm. in transverse and 1.1 mm. in longitudinal diameter, is in the caudal portion of the body, its aperture being ventro-subterminal and measuring 0.5 to 0.73 mm. in diameter. Encircling the aperture there is a prominent ring marked off from the general surface by a deep circular groove (figs. 152, 153, 161, 162). This projecting ring is seen in sections to be a portion of the acetabulum. The acetabulum is relatively much larger in this than in *Ps. stanleyii*, and its cavity is deeper.

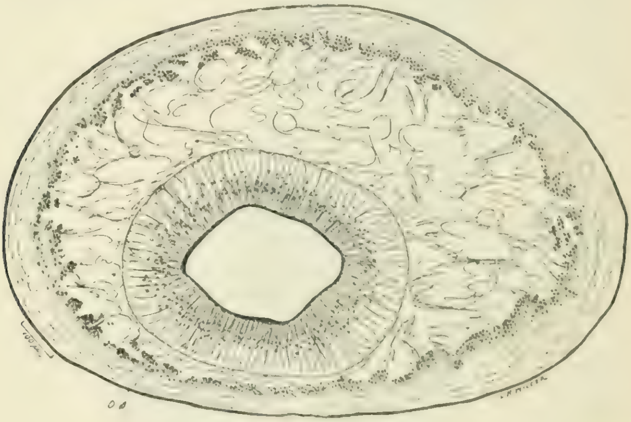

Fig. 156.

INTERNAL ANATOMY.

Digestive tract.—The cephalic extremity is pierced by a more or less circular aperture which measured 0.41 mm. and 0.36 mm., respectively, in 2 specimens. This oral aperture leads directly into a muscular sucker. As in the case of *Ps. stanleyii* the sucker may be divided for purposes of description into 2 portions, which are marked off by a constriction (or isthmus) at about its equator. The first or oral portion is of a somewhat globular form and measures 0.64 mm. in transverse and 0.52 mm. in dorso-ventral diameter; its lumen is of relatively considerable diameter, both transversely and ventrodorsally (fig. 156). Corresponding to the region of constriction referred to as the dividing line between the two portions of the sucker the lumen becomes a transverse slit and leads into the second, esophageal (or bulbous), portion of the sucker. This portion broadens out and, on each side of the origin of the esophagus, it extends caudad for a short distance of about 0.24 mm. in the form of a muscular bulb (figs. 154, 155). This portion of the sucker measures about 0.52 mm. in greatest dorso-ventral diameter, which is at about the equator of this portion of the sucker, and about 1 mm. in transverse diameter measured at the level of origin of the esophagus. Compared with the corresponding portion of the sucker of *Ps. stanleyii* this is relatively much larger in *Ps. collinsii.* From the ventral and from the dorsal wall of this portion of the sucker a transverse muscular ridge projects into the lumen of the sucker (figs. 155, 157) in a manner which strongly suggests the relation between the pharynx and oral sucker in *Fasciola hepatica.* The lateral projecting edges of these ridges serve as inner (median) boundaries of the dorso-ventrally-running slit-like lumen of the suctorial bulbs (fig. 158). In transverse section just above the level of origin of the esophagus the relation of the lumen of the sucker to that of its bulbs is such that combined they form a broad letter H (fig. 158).

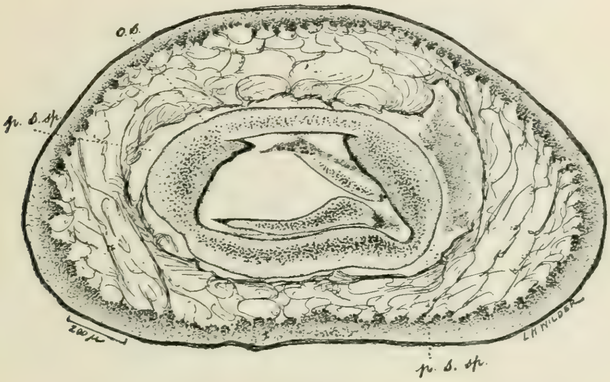

FIG. 157.

From the dorso-caudal aspect of the lateral suctorial prolongations or bulbs there springs on each side a pouch. These pouches are roughly globular in form, of relatively considerable dimensions. The right is a little larger than the left. They are in relation to the latero-dorsal aspects of the esophagus (fig. 159) and extend caudad to about the level at which the latter gives origin to the intestinal ceca. The pouches are sharply delimited by a cavity from the surrounding body parenchyma. Their walls consist of cells which, though large and resembling those of the body parenchyma, are much smaller than those of the latter. A considerable portion of the sucker, together with the bulbs, lies in a cavity strongly suggestive of a rudimentary body cavity and similar to the condition described for *Ps. stanleyii;* mesenterium-like bands are found dorsally and ventrally extending from the body wall to the digestive apparatus in question. The upper limit of this cavity is as in *Ps. stanleyii* at the level of the suctorial isthmus. At or slightly above the level of origin of the esophagus this cavity becomes definitely divided into two (fig. 158), which inclose the bulbs and extend caudad to a point slightly below (caudad of) the corresponding caudal margin of the pouch. The cavity contains a granular mass (fig. 158) resembling if not identical with the granular material encountered here and there in the excretory canals. The lumen of the second portion of the sucker is transversely elongate, more or less contracted ventro-dorsally, and extends laterally into the lateral suctorial prolongations; in these the lumen, though still slit-like, is directed ventro-dorsally. In its turn this dorso-ventral slit-like lumen is continued from its dorsal extremity into the lateral pouches. The lumina of the latter are irregular in form and variable in the degree of distention, both in different specimens and at different levels in the same specimen. The esophagus leaving the sucker, as already described, passes caudad, describing in its course a slight curve, with convexity ventrad, and at about the junction of the first with the middle third of the body length it terminates, giving origin laterally to the intestinal ceca. The intestines, as simple tubes, pass at first dorso-laterad and at the same time slightly caudad to a point about one-fourth the width from the lateral margins, where they bend almost directly caudad, describing a wavy course both transversely and ventro-dorsally, similar to that in *Ps. stanleyii*, and terminate by cecal extremities laterally of the acetabulum at about the level of the lower margin of the aperture of the latter. In their course caudad they markedly approach the median line at one point, namely, dorsad of the corresponding testis.

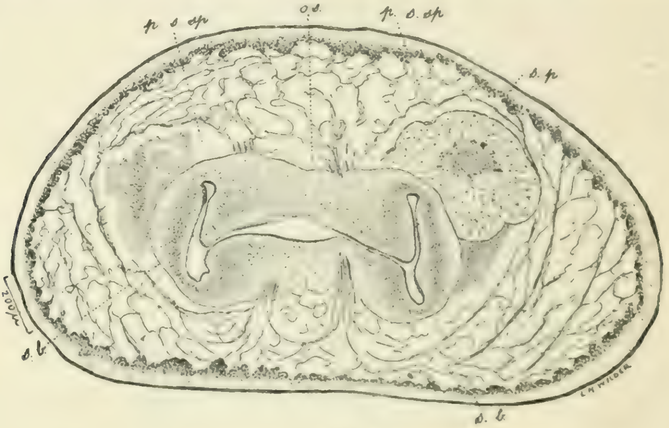

FIG. 158.

The lumen of the digestive tract from the oral margin to the intestinal ceca is lined by a cuticle-like layer in anatomical continuation with that of the body surface. It is thickest in the esophagus. In the region of the mouth in the esophageal portion of the sucker and in that part lining the lumen of the lateral suctorial prolongations or bulbs it is beset by numerous small conical papillæ. These papillæ were not observed in any other portion of the sucker or in the suctorial pouches. The intestinal lumen is lined by an epithelial cell layer.

Genital system.—As in *Ps. stanleyii,* the male and the female genital organs, except the vitellogene glands, are disposed in the median field, namely, between the intestinal ceca.

Male organs.—This worm is provided with two roughly globular, cauliflower-like (fig. 160) testes. They are disposed in one of the transverse diameters, (very) slightly (if at all) caudad of the equator of the worm. Each testis is a little nearer the median-sagittal plane than the corresponding lateral margin and much nearer the venter than the dorsum (figs. 154, 155, 160). From the dorso-median aspect of each testis there springs a duct, vas efferens, which passes more or less horizontally dorso-mediad, uniting with its fellow to form the vas deferens. The vas deferens is clearly differentiated into three portions, the first and second, vesicula and pars musculosa, being compactly coiled in the axial region of the body of the worm.

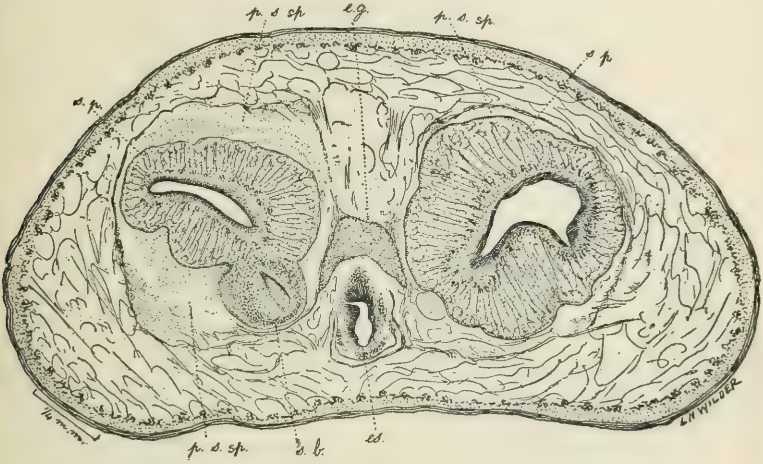

FIG. 159.

The vesicula is thin walled, but is not notably dilated; the musculosa, measuring about 112 μ in diameter, is provided with a relatively thick (37 μ) muscular wall; its terminal portion passes ventro-cephalad, and its wall becomes somewhat reduced in thickness as it passes into the third division (pars prostatica) of the vas deferens.

The latter is distinguished, as in other forms of this group, by being inclosed in a mass of nucleated cells; the prostatica is short and maintains the direction assumed by the distal portion of the pars musculosa, coming into close relation to the dorsal aspect of the terminal portion of the uterus; the prostatic cells cease a little before the male duct ends; this terminal portion appears to be very short and corresponds to the ductus ejaculatorius of the other forms. The ductus ejaculatorius appears to open just above the metraterm on the vertex of what may be regarded as a papilla forming the dorsal wall of a curved slit-like atrium. This atrium appears to communicate (?) with a smaller slit-like space immediately ventrad of it, from which there passes a slender duct, interpreted as the ductus hermaphroditicus, that opens on the vertex of a small genital papilla. The ductus hermaphroditicus, the two slit-like atria, the ductus ejactulatorius, and metraterm are inclosed in a somewhat globular mass of muscular fibers.

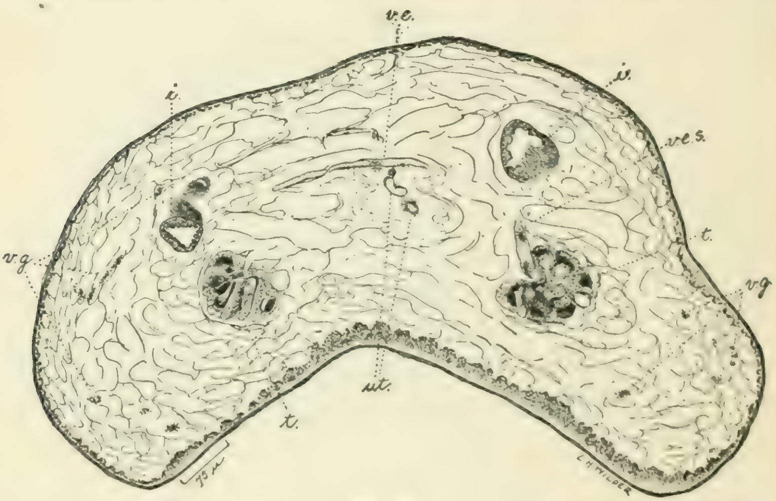

FIG. 160.

This description of the termination of the male and female canals and of the ductus hermaphroditicus is tentative, as it is based only on transverse sections of poorly preserved material, which are of difficult and doubtful interpretation.

Female organs.—In the posttesticular axial region of the body, but a little to the right of the median line and just above the upper margin of the acetabulum, is the ovary. Close to the caudo-mesial aspect of the latter is the slightly smaller shell gland (figs. 154, 155). The oviduct springs from the caudal aspect of the ovary and passes at first directly caudad for a very short distance, then it passes dorso-mediad and skirts the dorso-caudal aspect of the shell gland which it penetrates. Laurer's canal leaves the oviduct at a point just before the latter begins to skirt the shell gland; it passes caudo-dorsad, cephalad of the excretory vesicle, the dorsal aspect of which it gains, and ultimately it reaches the middle line of the dorsal surface at a point in a plane slightly cephalad of the equator of the acetabulum, and relatively some distance above the excretory pore. The vitellogene glands consist of sparsely scattered follicles in the lateral regions of the body (external to the intestinal ceca), appearing also ventrally and dorsally of the ceca. They extend longitudinally from the level of the middle of the esophagus to the level at which the intestinal tubes terminate. From each gland a duct passes transversely mediad in front of the corresponding intestine to unite with its fellow close to the ventro-caudal aspect of the shell gland. From their point of union (fig. 161) a smaller duct originates and passes dorsad and a little to the right, skirting the corresponding aspect of the shell gland which it penetrates at its caudo-dorsal aspect a little to the left of the point of penetration of the oviduct. Within the gland the vitello-duct unites with the oviduct, the duct resulting from their union forming the ootype and continuing beyond as the uterus.

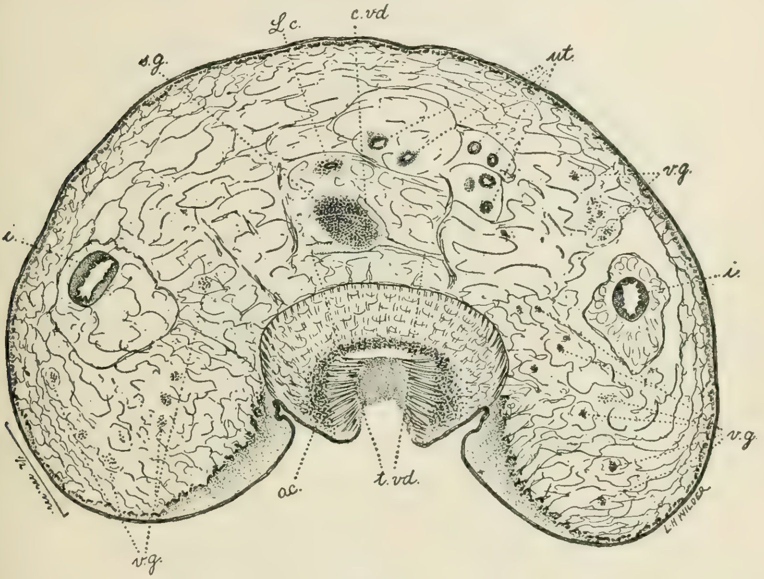

FIG. 161.

The uterus emerges from the ventral aspect of the shell gland, winds its way to the left, and then forms a number of coils in the axial body region to the left and dorsad of the ovary as it winds its way cephalad. Its windings cease at about the level of the cephalic aspect of the ovary; beyond this point the uterus pursues a direct, though slightly wavy, course ventro-cephalad to gain the ventral aspect of the coiled vas deferens, passing beneath and ventrad of the arch of union of the vasa efferentia. In the remainder of its course the uterus retains this relation to the ventral aspect of the male duct and, as has been described, appears to open immediately below the latter into the slit-like atrium (fig. 155.)

EXCRETORY SYSTEM.—The excretory system appears well developed. The excretory vesicle is in the caudal portion of the body dorsally of the acetabulum (figs. 155, 162). It extends caudad to about the level of the caudal margin of the acetabulum. At this point its lumen becomes reduced to a duct, with a narrow lumen but strong wall, which passes directly caudad and opens in the median line of the dorsum almost if not quite at the caudal extremity of the worm.

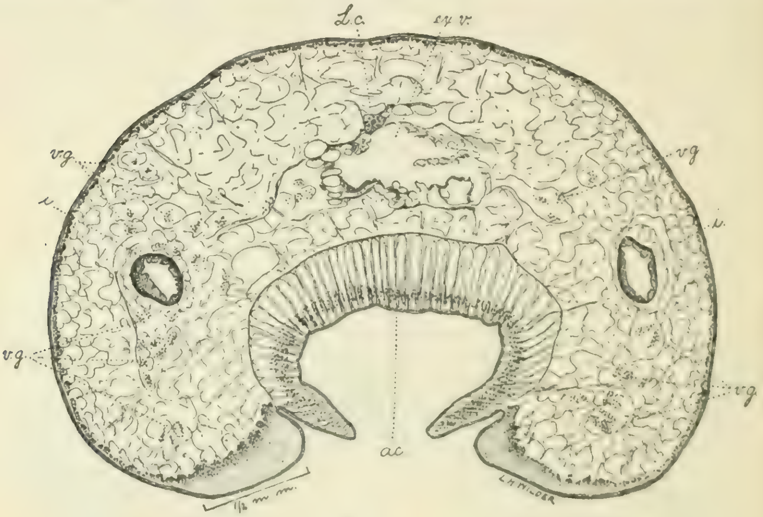

FIG. 162.

RELATION TO *Pseudodiscus stanleyii*.

Except for the difference in size, the two species are much alike in external appearance. A study of their internal anatomy brings out several points of difference. The esophageal portion of the sucker in *Ps. collinsii* is relatively much broader than that of *Ps. stanleyii*, and presents both a dorsal and a ventral transverse projecting ridge in its lumen; *Ps. stanleyii* presents a dorsal ridge only. The testes are actually and relatively much smaller in *Ps. collinsii* than in *Ps. stanleyii*, and furthermore they are more widely separated in *Ps. collinsii* than in *Ps. stanleyii*. The anatomy of the terminal portions of the genital canals appears to differ markedly in the two forms, though too much emphasis should not be placed on this because of the difficulty of interpretation of sections and of the limited and unsatisfactory nature of the material. The opening of Laurer's canal is much farther caudad in *Ps. collinsii* than in *Ps. stanleyii;* in the former it is a little above the caudal margin of the acetabulum, whereas in the latter it is only a little below the upper margin of the acetabulum. The acetabulum of *Ps. collinsii* is relatively much larger than of *Ps. stanleyii*, and its cavity is deeper. The ovary and the shell gland are at about the level of the upper margin of the acetabulum in *Ps. collinsii*, but distinctly above this level and nearer the level of the caudal aspects of the testes in *Ps. stanleyii*.

ILLUSTRATIONS.

FIG. 152–153.—Ventral aspect showing some of the variations in outline.

FIG. 154.—Frontal projection from transverse sections of specimen shown in fig. 152. Shows oral sucker (*o. s.*), suctorial bulbs (*s. b.*), suctorial pouches (*s. p.*), esophagus (*es.*), intestines (*i.*), genital pore (*g. p.*), testes (*t.*), vasa efferentia (*v. e.*), uterus (*ut.*), ovary (*ov.*), shell gland (*s. g.*) and acetabulum (*ac.*). *a–a, b–b, c–c, d–d, e–e, f–f, g–g,* planes of section. Slightly diagrammatic. Enlarged. Original.

FIG. 155.—Profile projection of specimen shown in fig. 152. Shows oral sucker (*o. s.*), left suctorial bulb (*s. b.*), suctorial pouch (*s. p.*), esophagus (*es.*), vas deferens (*v. d.*), uterus (*ut.*), ovary (*ov.*), shell gland (*s. g.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), excretory pore(*ex. p.*), and genital pore (*g. p.*). *a–a, b–b, c–c, d–d, e–e, f–f, g–g,* planes of section. Slightly diagrammatic. Enlarged. Original.

FIG. 156.—Transverse section through plane *a–a* figs. 154 and 155. Shows form of body and form of oral sucker (*o. s.*). Enlarged. Original.

FIG. 157.—Transverse section through plane *b–b* figs. 154 and 155. Shows oral sucker (*o. s.*) with ventral and dorsal ridges projecting into its lumen, and perisuctorial space (*p. s. sp.*) containing some granular coagulum. Enlarged. Original.

FIG. 158.—Transverse section through plane *c–c* figs. 154 and 155. Shows H-shaped lumen of oral sucker (*o. s.*) and suctorial bulbs (*s. b.*), the superior margin of the left suctorial pouch (*s. p.*) and the perisuctorial space (*p. s. sp.*) containing some granular coagulum. Enlarged. Original.

FIG. 159.—Transverse section through plane *d–d* figs. 154 and 155. Shows esophagus (*es.*), esophageal ganglion (*e. g.*), caudal portion of right suctorial bulb (*s. b.*), suctorial pouches (*s. p.*), and caudal prolongations of perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 160.—Transverse section at plane *e–e* figs. 154 and 155. Shows position and relation of the testes (*t.*), intestinal ceca (*i.*), arch of union of vasa efferentia (*v. e.*), origin of left vas efferens (*v. e. s.*), the uterus (*ut.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 161.—Transverse section at plane *f–f* figs. 154 and 155. Shows position and relation of caudal margin of shell gland (*s. g.*), union of transverse vitello-duct (*t. vd.*), the common vitello-duct (*c. vd.*), Laurer's canal (*L. c.*), uterus (*ut.*), vitellaria (*v. g.*), intestines (*i.*), and acetabulum (*ac.*). Enlarged. Original.

FIG. 162.—Transverse section at plane *g–g* figs. 154 and 155. Shows position and relation of intestines (*i.*), excretory vesicle (*ex. v.*), Laurer's canal (*L. c.*), vitellaria (*v. g.*), and acetabulum (*ac.*) with its projecting rim. Enlarged. Original.

HAWKESIUS, new subgenus.

SUBGENERIC DIAGNOSIS.—*Pseudodiscus* (p. 170): Esophageal wall with pronounced muscular thickening in caudal half. Testicular zones separate, nearly or quite abut, fields coincide.

TYPE SPECIES.—*Pseudodiscus* (*Hawkesius*) *hawkesii*.

PSEUDODISCUS HAWKESII (Cobbold, 1875) Stiles & Goldberger, 1910.

[Figs. 163 to 174.]

1875: *Amphist. hawkesii* Cobbold, 1875n, 818, 819 (in *Elephas indicus;* India); 1877e, 234; 1879b, 393, 396, 399; 1882, 238–240, fig. 8; 1883x, 515.—Braun, 1893a, 871, 905; 1893d, 466 (*hawkesi*).—Fischder., 1902a, 48 (*hawkesi*) (in *E. indicus;* India); 1903h, 489 (to *Pseudodiscus* by Sons., 1895).—Galli-Valerio, 1901c, 364 (in elephant).—Huber, 1896a, 579–580 (in elephant).—Looss, 1902m, 439 (*hawkesi*).—Mégnin, 1882v, 455.—Piana & Stazzi, 1900a, 511, 519–525, 529, figs. 12–14 (*hawkesi*); 1901, 416.

1893: *Amphist. hawkesi* Braun, 1893d, 466 for *hawkesii*.

1895: *Amphist. hawkesi* Sonsino, 1895, 182 for *hawkesii;* 1895, 187, fig. to *Pseudodiscus;* 1896, 310.

1895: *Amphist.* (*Pseudodiscus*) *hawkesi* (Cobbold, 1875) Sonsino, 1895, 187 (9), fig. 1.

1895: *Amphist.* (*Pseudodiscus*) *hawkesi* Sonsino, 1895, 9, fig. 1.

1896: *Pseudodiscus hawkesi* (Cobbold, 1875) Sonsino, 1896, 310.—Piana & Stazzi, 1900, 519 to *Amphist.*

SPECIFIC DIAGNOSIS.—*Pseudodiscus* (p. 170): Body 3.5 to 5 mm. long by 2 to 3 mm. broad; light buff in color (alcohol material); oval, oral pole bluntly rounded, tilted slightly ventrad, caudal pole very bluntly rounded, almost semicircular; lateral margins convex in transverse section, straight to convex longitudinally, dorsum convex, venter flat to slightly concave longitudinally and transversely; oral pole with slender digitate papillæ. Genital pore ventro median, postbifurcal, two-fifths of body length from oral margin with subhemispherical bulging. Acetabulum ventral at caudal end, oval to circular, 1.2 mm. in sagittal diameter, aperture 0.58 mm., dome 0.30 mm. thick; its margin projects and is separated from the body by circular groove; cavity rather shallow. Mouth subterminal; oral sucker constricted at equator into globular oral and a shorter broader esophageal portion; well defined bulbs absent, but there are 2 large pouches which extend caudad about to equator of esophagus; esophageal portion of sucker and the pouches lie in a perisuctorial cavity; esophagus arises from base of sucker, extends to caudal margin of oral third of body; the anterior half of esophagus about 90μ thick, with wall 22μ thick, its caudal half increased enormously (up to 330μ) in diameter by increase of muscular tissue; ceca long, extend in wavy course to or slightly beyond equator of acetabulum; their course is wavy and at equator of animal they approach toward median line. Excretory pore at caudal extremity;

excretory vesicle dorsal of acetabulum, extends from near shell gland to postacetabular zone.

Male organs: Testes preacetabular in same longitudinal median field, with separate or slightly overlapping zones, deeply lobate but not of cauliflower type, noticed in *Ps. stanleyii;* vasa efferentia arise from dorsal aspect, extend cephalo-dorso-laterad, then cephalad, uniting about 120μ cephalad of cephalic testis to form vas deferens; vesicula much coiled, musculosa coiled and well developed, prostatica and ejaculatorius short, the latter uniting with metraterm to form wide ductus hermaphroditicus, which discharges at genital pore; cirrus pouch absent.

Female organs: Ovary posttesticular, in testicular fields, in pre- and acetabular zone; shell gland postovarial; vitellaria, with small follicles, chiefly in extracecal area, apparently confined to cecal zone; uterus emerges ventrally from shell gland, passes in coils dorsad, caudad, then cephalad dorsally of ovary and testes, ventrocephalad under arch of vasa efferentia, to ductus hermaphroditicus; Laurer's canal long, extends from oviduct caudo-dorsad, dorsally of excretory vesicle to dorso-median line, opening slightly cephalad of caudal margin of acetabulum, cephalad of excretory pore.

Eggs: Not observed.

TYPE.—Unknown.

HABITAT.—Colon of elephant (*Elephas indicus*), India.

SOURCE OF MATERIAL.—The material at our disposal, consisting of 11 specimens, was kindly sent to us by Prof. Pietro Stazzi, of Milan, Italy. The specimens are presumed to be some of those collected by Piana and Stazzi (1900) from the colon of an elephant.

HISTORICAL REVIEW.—Cobbold (1875l, 736) originally named this species without giving any anatomical details, but discussing its possible effects upon the host. The worms were collected from the elephant and sent to him from Secunderabad, India. They were named in honor of the sender. In his second paper (1875n, 818, 819) no details were added. Later Cobbold (1877e, 234) refers to having noticed some papillæ in the acetabulum of this species (but the question arises as to whether he did not perhaps have before him specimens of *Amphist. papillatum* from the same host). Still later (1879a, 393, 396, 399) Cobbold refers to the worms in connection with the habit of dirt-eating among elephants, and states that he had also found *hawkesii* in an elephant which died in England.

Mégnin (1882v, 455) states that Cobbold had admitted in a letter that *Amphist. hawkesii* and *A. ornatum* represent two varieties of the same species.

Cobbold (1882a, 224, 238–240, 241, fig. 8) gives *Amphist. collinsii* var. *stanleyi* as synonym of *hawkesii*, but states that he thinks that *stanleyi* will eventually prove to be a good species. He publishes the following specific diagnosis of *hawkesii*, which really represents the first attempt at a detailed zoological description:

> Body of a pink color, smooth, plano-convex, finely wrinkled transversely, bluntly pointed and contracted in front, broadly rounded behind. Head surrounded by a few regular but not well-pronounced folds, armed with numerous small and extremely minute warty papillæ. Mouth terminal, circular. Ventral surface often slightly

depressed near the center, forming slight prominences on either side. Caudal sucker placed well forward, rather large, circular, with a broad lip and smooth concavity. Reproductive papilla small, situated nearly midway between the mouth and upper margin of the caudal sucker. Length, on the average, three-eighths of an inch; the longest specimen seven-sixteenths of an inch. Breadth one-fourth of an inch. Hab., large intestines of *Elephas indicus*.

Cobbold's figure 8 does not add any essential details.

Cobbold (1883x, 515) again refers to this species incidentally, but gives no further details. Neither are any additional data given by Braun (1893a, 874, 905; 1893d, 466), Huber (1896a, 579–580), Fischœder (1902a, 48; 1903h, 631), or Looss (1902m, 439), none of whom examined this species.

Sonsino (1895, 4) considers *hawkesii* as identical with *collinsii*, transfers the species to *Pseudodiscus*, and gives the following specific characters:

> Color rosso carnicino come generalmente tutti gli Amfistomidi di mammiferi. 10 mm. by 6 mm. Corpo allungato, convesso pianeggiante, sensa peduncolo distinto, con leggière striscie trasverse e coll' estremo anteriore ristretto, ma ottuso e l' estremo posteriore arrontondato. Bocca terminale e circolare. Superficie ventrale spesso alquanto depresse verso il centro, dando così apparenza di superficiale escavazione, con due leggière prominenze laterali corrispondenti probabilmente alle due masse testicolari. Ventosa posteriore subterminale, larga, circolare con margine grosso e con cavità liscia. Papilla genitale piccola a mezza distanza tra la bocca e il margine superiore della ventosa posteriore.

Later, Sonsino (1896, 310) merely mentions the worm.

Piana and Stazzi (1900, 520-525) described specimens of worms determined as *Amphistomum hawkesi* found in the colon of an elephant autopsied in Milan. Their diagnosis reads:

> Corpo di color rosso carnicino, oblungo, convesso nel superficie dorsale ed escavato a doccia in quella ventrale. Estremità arrotondate, quella posteriore piu larga della anteriore. Lunghezza del corpo, in tutti gli individui esaminati, no superiore a 6 mm.; larghezza corrispondentemente al terzo anteriore del corpo 2.70 mm., corrispondentemente al terzo posteriore 3.70 mm. La superficie cuticolare, guardata con lente, appare irregolarmente striata nel senso tras-versale. Sul margine della parte anteriore del corpo, alquanto verso la superficie ventrale, si trova la bocca oblunga in senso verticale circondata da una piccola ventosa del diametro di 0.18 mm., la quale è limita da un orlo della larghezza di 0.10 mm. Sulla superficie ventrale a livello del limite tra il terzo anteriore e il terzo mediano delle larghezza del corpo, si trova il poro genitale, in forma di un orifizio oblungo in senso trasversale, lungo 0.25 mm. e largo 0.15 mm. Sempre sulla superficie ventrale, alla distanza di 1.80 mm. della ventosa boccale, e quindi nella parte posteriore del corpo, si trova la ventosa posteriore, la quale è circondata da un orlo largo circa 0.17 mm. ed ha un orifizio del diametro di 0.40 mm.

Of the internal anatomy they recognized the "pharynx" (oral sucker), with a diverticulum each side, an esophagus 0.7 mm. long with posterior muscular thickening, and 2 long wavy ceca which end in the suctorial zone; the ovary and "vitellogene gland" [= shell gland] are located near the acetabulum; the uterus is flexuous and extends cephalad to the genital pore; dextral of the "female pore" there are seen structures indicative of the cirrus, vas deferens, and the 2 testes, but they figure (fig. 14) 2 pairs of testes, 1 pair cephalad of the other, zones separate, fields coincide.

Piana and Stazzi point out that their specimens are smaller than Cobbold's, but explain this fact by assuming that they are dealing with young worms.

We accept the specific determination of their worms as *Amphist. hawkesii* on the principle that when an author claims to have identified a species his identification is to be assumed to be correct until proved to be incorrect. At the same time we may be permitted to recall the fact that recent work on trematodes has caused a great many surprises and it is not by any means excluded that if Cobbold's original specimens can be found, the material here accepted as *hawkesii* may eventually prove to be a distinct species.

In order to avoid a troublesome nomenclatural difficulty which frequently arises, and in regard to which authors are at present divided in opinion, we would state that should the original material of *hawkesii* prove to be distinct from the material here described, the subgenus *Hawkesius* is based upon the material now on hand, without any reference to characters which may or may not be present in the type of *hawkesii*. In other words, the species represented by U.S.P.H. & M. H.S. no. 10545 is the type of *Hawkesius*.

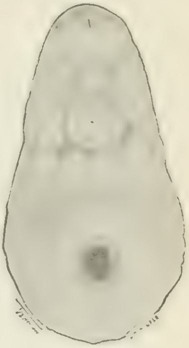

FIG. 163.

EXTERNAL CHARACTERS.

SIZE.—The alcohol-preserved specimens varied in length between 3.5 and 5 mm., and in greatest width between 2 and 3 mm.

COLOR.—The worms are of a light buff tint.

FORM.—The bottle in which the specimens were sent to us was found on arrival to be broken and the specimens dried out. They were put into 70 per cent. alcohol in which they regained to a considerable extent their original form, but they are still shrunken and deformed to a considerable degree (figs. 163, 164). They suggest *Ps. collinsii* in form, but appear more elongate and with the oral pole relatively more acutely pointed.

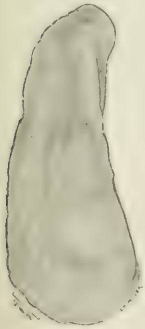

FIG. 164.

The dorsum is convex both longitudinally and from side to side; the venter is flat or slightly excavate in both the longitudinal and transverse directions, the concavity being best defined in the caudal half of the venter. The oral pole is bluntly rounded, presents a slightly bulbous appearance and is tilted to a variable degree ventrad. The caudal pole is broad and rounded from side to side. The lateral margins are convex in transverse section and straight or but slightly curved longitudinally.

SURFACE.—The cuticle is marked on its ventral surface by fine transverse sulci. These sulci are most clearly defined in the region between the genital pore and acetabulum. Around the oral pole there are slender digitate papillae (fig. 172), some of which measure 90μ in length when measured in sagittal sections.

Genital pore.—In about the median line of the ventral surface, about two-fifths of the body length from the oral margin, is a distinct subhemispherical ventral bulging (figs. 163, 169) of variable extent, measuring in sections of one specimen 0.375 mm. in longitudinal diameter. At the vertex of this bulging is the genital pore. This pore measured 150μ in longitudinal diameter in one sectioned specimen. It leads into an irregularly cylindrical chamber into the lumen of which there arises from its dorsal wall the genital papilla (fig. 165).

There was observed in several of the specimens still another ventral bulging. This is elongate, in the median longitudinal line (fig. 163) and extends from a little below the bulbous oral extremity to almost the genital bulging. This elongate bulging is found in sections to correspond to the esophageal bulb (fig. 168). In the median line of the caudal portion of the venter the aperture of the acetabulum presents itself. It is irregularly oval or circular in form encircled, as in *Ps. collinsii* and *Ps. stanleyii*, by a rim which is marked off from the general surface by a deep narrow groove. On each side of this aperture the venter of the worm bulges out so that it appears to be in a more or less well-marked fairly broad vertical groove (fig. 163).

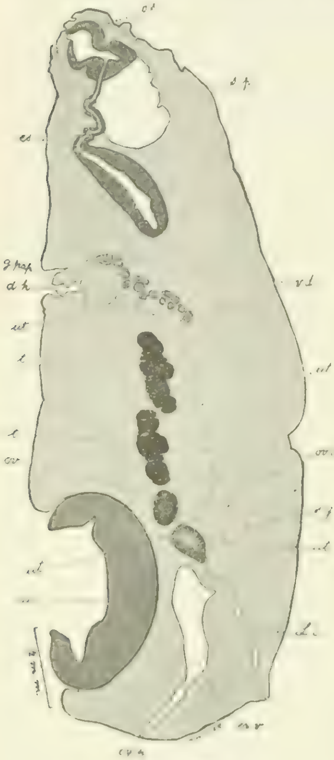

FIG. 165

Acetabulum.—The acetabulum is in the caudal portion of the body, and, as already stated, its aperture is on the ventral aspect of the caudal extremity. Measured in sagittal sections of one specimen the vertical diameter was 1.20 mm., with an aperture of 0.58 mm. in the same diameter, with a thickness of dome of 0.30 mm. As in *Ps. collinsii* and in *Ps. stanleyii* the acetabular aperture is encircled by a projecting ring (figs. 163, 165) marked off from the general body surface by a deep narrow groove. This ring is formed by part of the acetabulum itself.

INTERNAL ANATOMY.

Digestive tract.—The cephalic extremity is pierced by the mouth which is somewhat elliptical in form and directed dorso-ventrally. The aperture of the mouth leads directly into the oral sucker; this appears to be divided into two portions by a circular constriction at about its equator. The first or oral portion appears somewhat globular in form. The form of the second or esophageal portion can not be made out accurately from the material at our disposal. In a general way, however, it is shorter but broader than the oral portion. The increase in the transverse diameter is preparatory to the formation of lateral suctorial pouches which project caudad and laterad of the point of origin of the esophagus. They are irregularly globular in form and extend caudad on each side and dorsally of the esophagus (figs. 165, 167, 168) to about the equator of the latter. A well-defined suctorial bulb, such as was described for *Ps. collinsii* and *Ps. stanleyii*, is not present. The suctorial walls are muscular (figs. 166, 172), but the arrangement of the muscular bundles is less compact than in *Ps. collinsii*. The structure of the pouch wall (figs. 167, 168) is distinctly less muscular than that of that portion of the sucker from which the pouches project and the transition in structure from one to the other can readily be made out. The cell structure of the pouch walls is loose but the cells are decidedly smaller than those of *Ps. collinsii*. The lumen of the sucker and pouches is lined by a thin cuticle-like layer. That of the second portion of the sucker is closely beset by small conical papillæ. A space around the caudal portion of the sucker and the pouches similar to that in *Ps. collinsii* is present (figs. 166, 167, 168). The esophagus springs from the ventral aspect of the base of the sucker. For about one-third its length it passes in a slightly wavy course caudad, then turns rather abruptly and passes directly dorso-caudad to a point in a transverse plane at about the junction of the first with the equatorial third of the body and about midway between venter and dorsum, where it gives off the intestinal ceca. The walls of the first portion of the esophagus measured in transverse sections of one specimen were about 22μ in thickness, the diameter of the esophagus at the same point being about 90μ. In the second portion of the esophagus the walls become greatly thickened

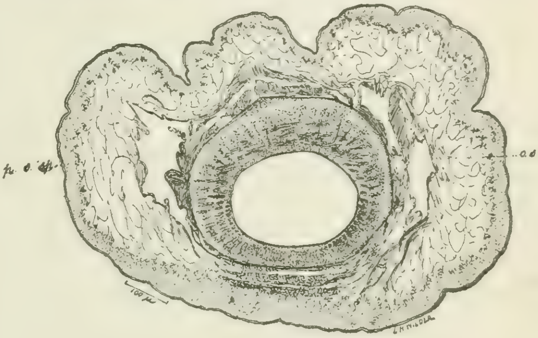

Fig. 166.

by the development of its muscular layers. In this portion three layers can readily be made out, an internal and an external of longitudinal, and a middle, very thick layer, of circular fibers (figs. 172, 173). This portion of the esophagus measured, in a sagittal section of one specimen, was 750μ long with a maximum dorso-ventral diameter of 330μ and with a thickness of wall of between 90μ and 105μ. The esophagus is lined by a fairly thick cuticle-like layer.

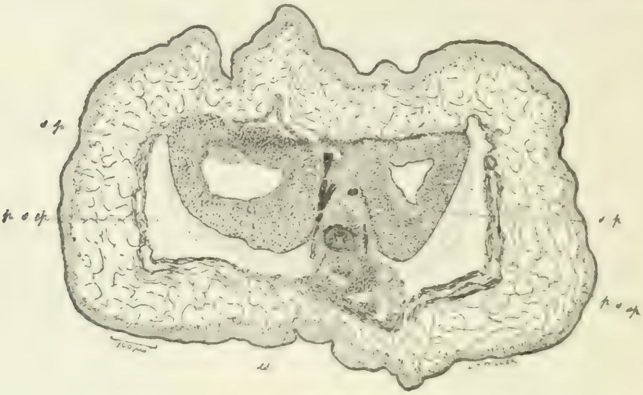

FIG. 167.

The intestinal ceca pass laterad, at first almost at right angles with the esophagus, then describing a gentle curve they pass caudad in a ventro-dorsal wavy course apparently similar to that in *Ps. collinsii* (fig. 174). At about the level of the equator of the animal the ceca approach close to the corresponding dorso-lateral aspect of the superior testis. This is also the point where in their course the intes-

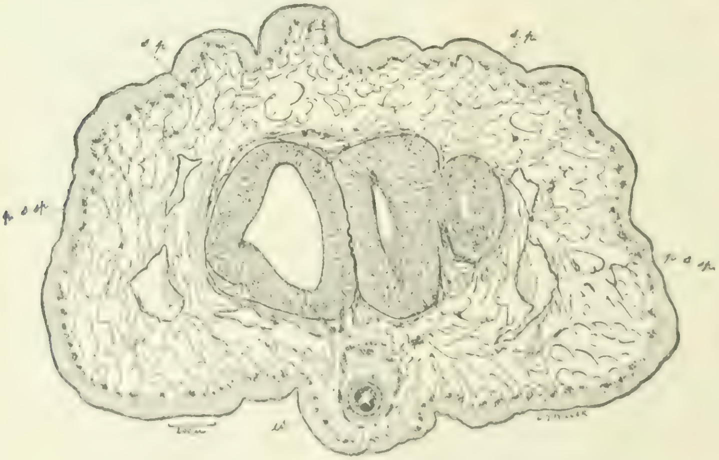

FIG. 168.

tines come nearest together. A similar peculiarity has been noted in *Ps. collinsii* and *Ps. stanleyii*. The intestines extend caudad to or slightly beyond the equator of the acetabulum. In transverse sections the ceca are irregularly circular in outline and are more or less constricted at irregular intervals, causing considerable variation in diameter. The lumen is lined by an epithelial cell layer.

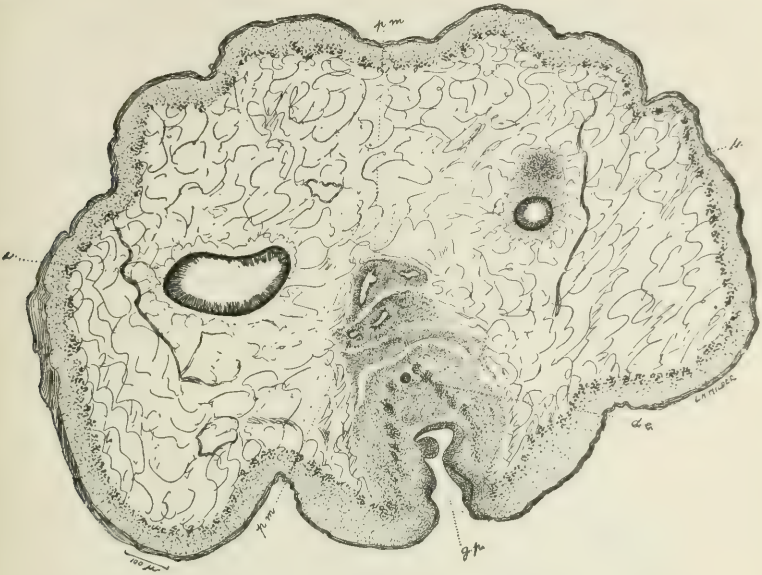

Fig. 169.

Genital system.—With the exception of the vitellogene glands the genital organs are disposed in the intercecal space.

Male organs.—The two testes are in the axial region of the body, somewhat nearer the venter than the dorsum. They occupy about two-ninths of the body length in the zone immediately cephalad of the acetabulum. They are placed one directly caudad of the other, their zones either overlapping very slightly or separated by a very narrow interspace. The two testes are of about the same size; measured

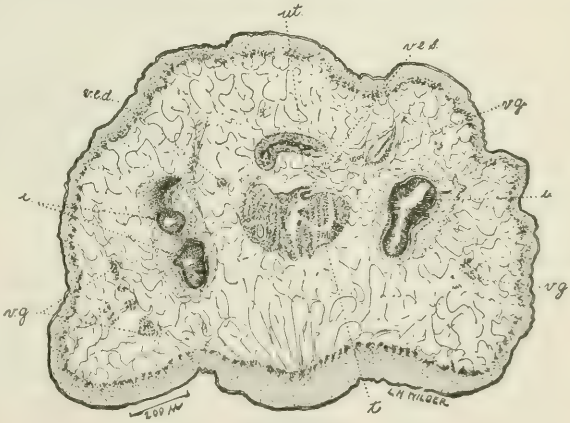

Fig. 170.

from a series of transverse sections of one specimen the superior testis was 420μ long by 365μ wide by 210μ thick, while the caudal testis was 400μ long by 390μ wide by 180μ thick. Each testis is divided into numerous lobes by deep infoldings of its inclosing membrane. The testes are not quite of the cauliflower-type, such as those of *Ps. collinsii* and *Ps. stanleyii.* From the dorsal aspect of each testis there emerges a vas efferens (figs. 170, 171). After emerging from the testes the vasa efferentia tend cephalo-dorsad and away from the median line, the vas from the superior testis going to the left and that from the inferior (or caudal) to the right. After reaching a point near the mesial aspect of the corresponding intestine the vasa pass directly cephalad until they reach a plane slightly (about 120μ) cephalad of the superior aspect of the superior testis, when each curves inward to unite with its fellow to form the vas deferens. In so doing the vasa efferentia form a transverse arch, beneath which the uterus passes as it ascends cephalo-ventrad. The vas deferens presents in the first part of its course a much coiled vesicula, which is succeeded in the second part by a coiled, well-developed musculosa. The latter gives place to a short prostatica. The terminal portion of the vas deferens is a short narrow duct, the ductus ejaculatorius, which unites at the base of the genital papilla with the terminal portion of the uterus to form the ductus hermaphroditicus (fig. 165, *d. h.*). The latter is a wide duct which pierces the genital papilla to open on the vertex of the latter at the porus hermaphroditicus.

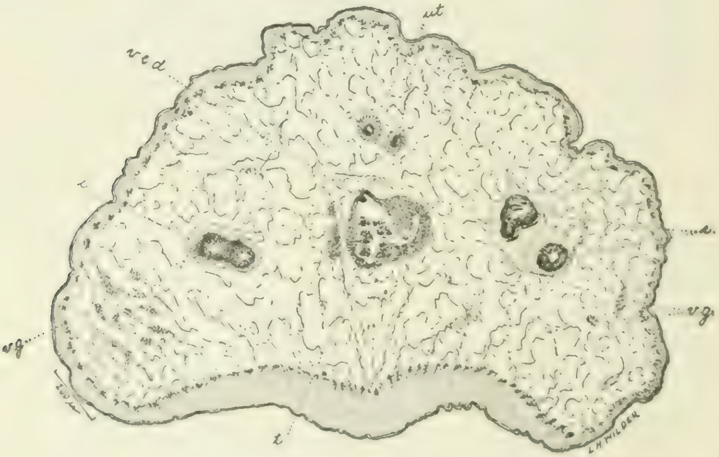

FIG. 171.

Female organs.—The ovary lies posttesticular, a little to the left of the median sagittal plane, close to the acetabulum, and in or immediately caudad of the transverse plane of its superior margin. The oviduct springs from the dorsal aspect of the ovary and passes caudolorsad, skirting the dorsal aspect of the shell gland and giving off Laurer's canal close to the dorso-caudal aspect of the latter. The nain duct then penetrates this aspect of the shell gland. The shell gland, apparently somewhat larger than the ovary, lies immediately caudo-dorsad of the latter. It is, as just described, penetrated on ts caudo-dorsal aspect by the oviduct; on its caudal aspect it is penetrated by a duct which is interpreted as the vitello-duct. The union between these ducts is not satisfactorily made out in our preparations, but no doubt it takes place to form a fairly distinct ootype, the continuation of which emerges from the ventro-cephalic aspect of the gland as the uterus (fig. 165). After emerging, the uterus turns to the right and dorsad, then dips caudad dorsally of the shell gland and in the axial region of the worm, forming some coils, then turns cephalad describing coils as it ascends between the dorsum and the testes. It passes ventro-cephalad over the superior testis and beneath the arch of union of the vasa efferentia to gain the ventral aspect of the coiled vas deferens. It retains this relation in the remainder of its course to the base of the genital papilla, where it

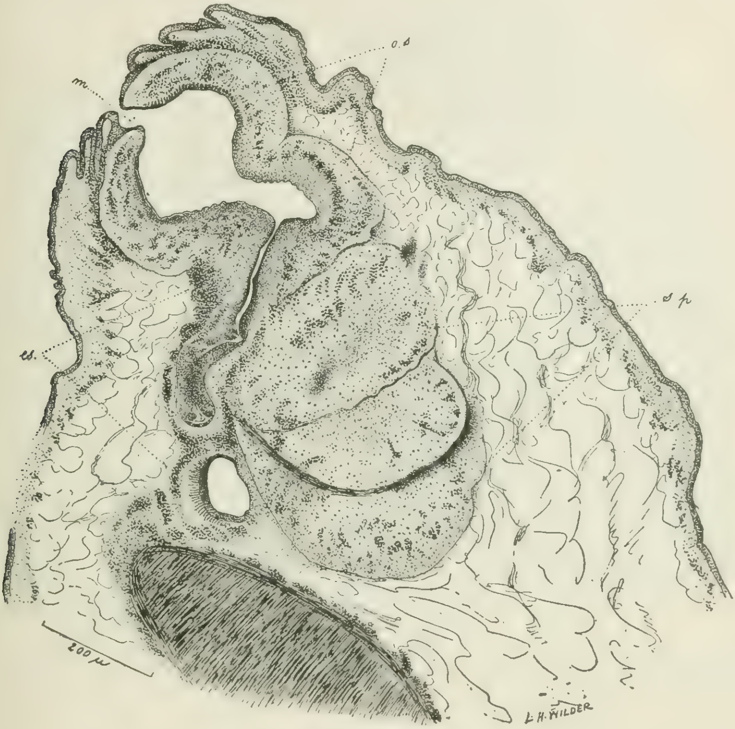

FIG. 172.

unites with the ductus ejaculatorius in the formation of the ductus hermaphroditicus.

Laurer's canal passes caudo-dorsad dorsally of the excretory vesicle to open in about the median line of the dorsum, cephalad of the excretory pore and slightly cephalad of the level of the caudal margin of the acetabulum.

The vitellogene glands, consisting of small insignificant follicles, are longitudinally disposed in the extracecal area, their caudal portions extending, however, into the cecal and to a slight extent into the intercecal area. Longitudinally they do not appear to extend outside of the cecal zone, but this point can not be made out from our preparations as satisfactorily as is desirable.

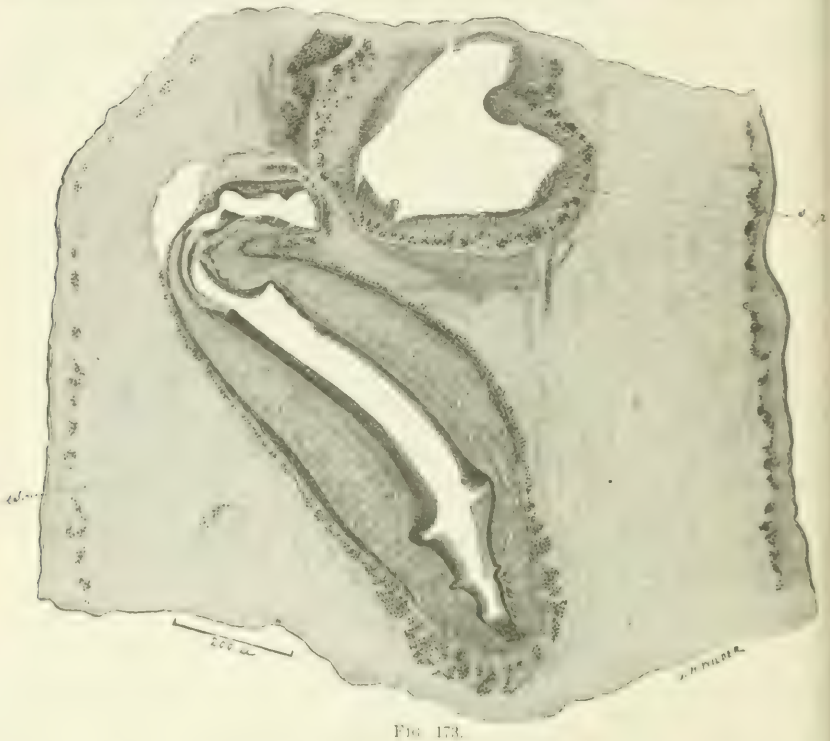

FIG. 173.

EXCRETORY SYSTEM. The excretory vesicle lies close to the dorsal aspect (the dome) of the acetabulum (fig. 165, *ex. v.*). It begins close to the ventro-caudal aspect of the shell gland and extends to slightly above the caudal margin of the acetabulum, beyond which point it is continued as a relatively thick duct to open in about the median line of the caudal extremity. This duct is inclosed in a well-marked layer of nuclei, probably of cells, the bodies of which, however, are not satisfactorily discernible. The excretory canals can not be satisfactorily followed in our preparations.

ILLUSTRATIONS.

FIG. 163.—Ventral aspect. Enlarged. Original.

FIG. 164.—Profile view of same. Enlarged. Original.

FIG. 165.—Diagrammatic sagittal section, showing oral sucker o. s.), suctorial pouch (*s. p.*), esophagus (*es.*), testes (*t.*), vas deferens v. d.), uterus (*ut.*), ovary (*ov.*), oviduct (*ov. d.*), shell gland (*s. g.*), Laurer's canal (*L. c.*), excretory vesicle (*ex. v.*), excretory pore (*ex. p.*), genital papilla (*g. pap.*), ductus hermaphroditicus (*d. h.*), and acetabulum (*ac.*). Enlarged. Original.

FIG. 166.—Transverse section (about through equator of oral portion of sucker, *o. s.*) to show its form and the perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 167.—Transverse section (at level of origin of esophagus) to show position and relations of suctorial pouches (*s. p.*) and beginning of esophagus (*es.*); also shows extension of perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 168.—Transverse section at beginning of bulbous portion of esophagus to show position and relation of fundi of suctorial pouches (*s. p.*) to this portion of the esophagus (*es.*). Shows also extension of perisuctorial space (*p. s. sp.*) and molding of venter over the esophagus. Enlarged. Original.

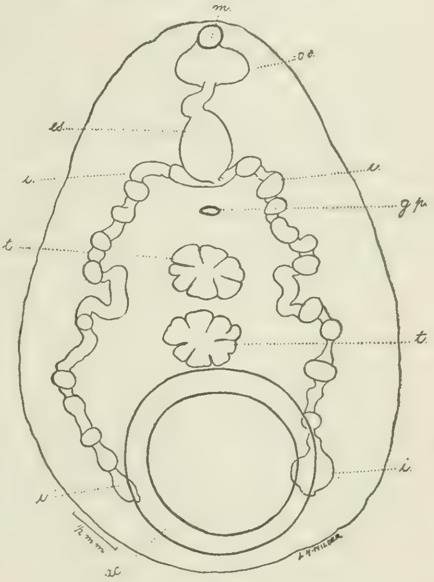

FIG. 174.

FIG. 169.—Transverse section at level of genital pore, showing subhemispherical genital bulging, genital pore (*g. p.*), pars musculosa (*p. m.*), ductus ejaculatorius (*d. e.*), and intestinal ceca (*i.*). Enlarged. Original.

FIG. 170.—Transverse section at level of origin of left vas efferens (*v. e. s.*) from superior testis (*t.*). Shows also position and relations of intestinal ceca (*i.*), uterus (*ut.*), right vas efferens (*v. e. d.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 171.—Transverse section at level of origin of right vas efferens (*v. e. d.*) from caudal testis (*t.*). Shows also position and relations of intestinal ceca (*i.*), uterus (*ut.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 172.—Sagittal section through oral extremity. Shows mouth (*m.*), oral sucker (*o. s.*), and first portion of esophagus (*es.*); also shows mesial wall of suctorial pouch (*s. p.*). Enlarged. Original.

FIG. 173.—Sagittal section through second portion of esophagus (*es.*); shows also portion of suctorial pouch (*s. p.*). Enlarged. Original.

FIG. 174.—Diagram to show topography of digestive tract. *ac.*, acetabulum; *g. p.*, position of genital pore; *es.*, esophagus; *i.*, intestinal ceca (showing constrictions and dilatations); *m.*, mouth; *o. s.*, oral sucker (and pouches); *t.*, testes; Enlarged. Original.

WATSONIUS, new genus.

GENERIC DIAGNOSIS.—*Cladorchiinæ* (p. 169): Body pyriform. Ventral pouch absent. Acetabulum ventral or ? ventro-subterminal; very large, margins projecting; aperture small. Genital pore prebifurcal, without sucker; ductus hermaphroditicus apparently absent. Excretory pore caudad of vesicle, in acetabular zone, caudad of pore of Laurer's canal. Oral sucker with a pair of latero-caudal irregularly globular suctorial pouches; esophagus with distal thickening of muscular layer (esophageal thickening); ceca long, not wavy, end postequatorial, posttesticular, in acetabular zone.

Male organs: Testes 2, lobulate, smaller than acetabulum, fields nearly or quite coincide, zones abut to slightly overlap, preovarial, not far removed from acetabulum, in equatorial and caudal thirds; musculosa not enormously developed; cirrus pouch absent.

Female organs: Ovary and shell gland immediately posttesticular; vitellaria extend about from bifurcal zone to slightly postcecal, into acetabular zone; uterus interceecal, in part posttesticular; Laurer's canal cephalad of excretory vesicle.

TYPE SPECIES.—*Watsonius watsoni* (Conyngham, 1904).

WATSONIUS WATSONI (Conyngham, 1904) Stiles & Goldberger, 1910.

[Figs. 175 to 189.]

1904: *Amphist. watsoni* Conyngham, 1904, Aug. 13, 464; Aug. 27, 355; 1905, Sept. 8; 1902, Sept. 17, 663, figs. 1-2 (in *Homo*, Africa); 1905, Sept. 29, 1480; 1905, Oct. 8, 710.—Shipley, 1905, 8 to *Cladorchis*.

1905: *Cladorchis watsoni* (Conyngham, 1904) Shipley, 1905, 129-135, pl. 4, figs. 1-10 (in *Homo*, Africa); 1905, 1-9, pl. 4, figs. 1-10; 1905, Apr., 205; 1905, Apr. 8, 950; 1905, Nov. 2, 1298; 1905, 9 pp., 10 figs.—Braun, 1908, 4 ed., 175-176, figs. 125-126.

SPECIFIC DIAGNOSIS.—*Watsonius* (p. 212): Body 8 to 10 mm. long, by 4 to 5 mm. in maximum breadth, by 4 mm. thick; fresh specimens reddish-yellow, translucent, gelatinous, preserved specimens dark slate to dirty brown in color, pyriform, greatest diameter about at border of equatorial and caudal thirds, tapers cephalad to about 2.5 mm.; tapers more rapidly caudad so that the caudal extremity is very bluntly rounded; lateral margins convex on ventral view, venter somewhat flattened and slightly indented posteriorly at margin of acetabulum; venter surrounded by an elevated ridge and bulges posteriorly [transverse sections are too irregularly contracted from preservation to permit of safe interpretation]. Surface with transverse ridges, coarser, and better defined ventrally. Genital pore ventro-median, "about 2 mm. from the oral sucker," or rather prominent, about one-fourth of body length from anterior end; about at equator of esophageal zone and in zone of suctorial pouches. Acetabulum ventro-subterminal (or ventral?), very large, over 1 mm. in diameter, its free margin projects considerably; aperture small. Mouth ventro-subterminal in a dorso-ventral groove (contraction?), with digitate papillæ; oral sucker sunken in body, very large, about one-fifth as long as body, attains 1.2 mm. in transverse and 1.1 mm. in dorso-ventral diameter, with a pair of latero-caudal irregularly globular suctorial pouches, which extend about halfway to bifurcal zone; esophagus slightly longer than oral ucker, distinctly bent, its convexity ventrad, its muscular wall thicker in its caudal alf; ceca extend into fifth zone, ending in anterior half of acetabular zone, their lumina ompressed laterally so that their dorso-ventral diameter is about 5 times as great as heir transverse diameter. Excretory pore dorsal, apparently slightly sinistrad of nedian line, in zone of aperture of acetabulum; excretory duct thick-walled; excretory esicle relatively small, dorsal of acetabulum, extends about from plane of transverse itello-ducts to equator of acetabulum.

Male organs: Testes large, deeply notched (lobulated), each about one-seventh as long as body, one caudad of the other, in median line, fields coincide, zones overlap very slightly; each vas efferens springs from dorso-cephalic aspect [point of union not definitely traced]; vas deferens consists of vesicula seminalis intricately coiled and dilated, pars musculosa relatively short and not coiled, a dilated portion (corresponding to pars prostatica), in which no prostatic cells were found, a relatively long, narrow ductus ejaculatorius which opens on genital papilla, cephalad of metraterm, into papillated genital atrium; terminal portion is surrounded by a muscular mesh which forms a genital bulging; true cirrus pouch absent.

Female organs: Ovary slightly sinistrad (but apparently touches median line), dorso-caudad of posterior testis, dorso-cephalad of acetabulum; shell gland dorsad of ovary; vitellaria with moderate number of well-developed follicles, ventro-laterad of ceca, in extracecal and cecal area, extend from bifurcal zone into postcecal zone about to equator of acetabulum; uterus passes from shell gland ventro-dextrad into acetabular zone not quite to end of ceca, bends cephalad, runs in coils dorsally of testes, then in rather straight to sinuous course ventrally of vas deferens to its opening caudad of male opening; apparently no ductus hermaphroditicus present; Laurer's canal opens apparently in dorso-median line, very slightly caudad of cephalic limit of acetabular zone.

Eggs: Eggs oval, 122 to 130μ long by 75 to 80μ broad.

TYPE.—(?). Cotype U.S.P.H.&M.H.S. 10720.

HABITAT.—Jejunum and duodenum of man (*Homo*); German West Africa.

SOURCE OF MATERIAL.—We are indebted to the kind courtesy of Dr. A. E. Shipley, to whom we desire to express our sincerest thanks, for the loan of a series of transverse sections of this worm.

HISTORICAL REVIEW.—At a meeting of the Section of Tropical Diseases of the British Medical Association, on July 27, 1904, Dr. H. C. Conyngham, demonstrator at the London School of Tropical Medicine, presented a paper entitled "A new trematode of man (*Amphistoma watsoni*)." This paper was abstracted in the Lancet, 1904, August 13, page 464, and Journal of Tropical Medicine, 1904, August 15, page 252. The full paper, which appeared in the British Medical Journal, September 17, page 663, reads as follows:

A NEW TREMATODE OF MAN (*Amphistoma watsoni*).

> Last February Doctor Watson, of Northern Nigeria, sent six curious trematodes from the small intestine of a negro, who had died of starvation and diarrhea, to the London School of Tropical Medicine. These proved to be a species of *amphistome*, totally unlike the *Gastrodiscus hominis* of Lewis—so far the only one of that genus found in man—and also unlike any hitherto described as occurring in animals. A specimen was sent to Professor Blanchard, of Paris, who very kindly examined it and reported that he considered it a new species. Doctor Watson sent some clinical notes of the case; they are as follows;
>
> The patient—one of a gang of freed slaves, all of whom were in a pitiable condition due to starvation—was brought from Adamawa, German West Africa, to Zola, Northern

Nigeria. He was found to be suffering from diarrhea, and was admitted to hospital, where he died the same night. His stools were numerous, watery, and of a bilious color, but containing no blood or mucus. In the stools were found many reddish-yellow, translucent, gelatinous, oval bodies (the trematodes). Necropsy revealed the spleen small, hard, and black. In the stomach some undigested milk was found. The duodenum and upper part of the jejunum were found full of these oval bodies, some of which were alive and adherent. The mucus membrane showed no hemorrhages, but appeared to be slightly red. The other parts of the bowel, as also the other organs, were normal. A few of the bodies were seen lying in the large intestine. The patient was extremely fond of eating raw meat.

The animals are pear shaped, flattened ventrally and slightly indented posteriorly at the margin of the posterior sucker, but owing to the preservative used they have shrunk considerably and are now of a dark slate color. The anterior sucker in most of the specimens is retracted and lies at the bottom of a sulcus, which is terminal and ventral; the posterior sucker is very large, its cavity measuring over 1 mm. across; it is subterminal and ventral. The genital pore lies about a quarter of the length of the parasite from the anterior end and is rather prominent. The cuticle of the body is marked with transverse ridges, these being coarser and better defined on the ventral surface; the latter is flattened, surrounded by an elevated ridge and bulges posteriorly.

The worms measure 8 mm. long, 5 mm. at point of greatest breadth, this tapering gently anteriorly to 2.5 mm.; their greatest thickness is about 4 mm.

The genital pore lies 2 mm. from the anterior sucker. The ova as seen in the uterus are oval and measure 130μ by 75μ. It was found impossible to clear the specimens sufficiently to make out definitely their internal anatomy, but the general arrangement seems to be like that of the *Amphistoma conicum* (Zeder).

That these parasites may have been the cause of death is not at all unlikely, when it is considered that the larger part of the small intestine contained a great number of them and that at least one other species of the same genus causes serious sickness in the higher animals, namely, the amphistome of Collins in India, causing *masuri*, a condition of severe intestinal irritation in horses; and another, the *Gastrodiscus* of Sonsino, is supposed to cause death in horses and mules in Egypt, Senegal, and Guadeloupe. Another trematode of a different genus, the *Fasciolopsis buski*, inhabitating the small intestine of man, has been credited with causing intestinal irritation and typhoid-like symptoms, and this is occasionally followed by death.

These worms are, therefore, a new parasite of man, probably causing serious intestinal disturbance, diarrhea, marasmus, and death. Whether their distribution is limited, which is likely, remains to be seen; but by a careful examination of feces for ova, or adults in cases of diarrhea, it may be found again in at least that part of Africa in which the patient resided.

It is not at all probable that the eating of raw meat, which Doctor Watson notes, has anything to do with their introduction into the body, as parasites of that genus are, as a rule, ingested in the larval or cercarial form, encysted on some vegetable substance.

I would suggest that the name *Amphistoma watsoni* be given to the interesting parasite.

Shipley (1905, 3-9, pl. 4, figs. 1–10) gave the history of this trematode, as furnished by Doctor Watson, as follows:

The patient was a Pagan who had come from Adamawa, German West Africa—one of a gang of freed slaves brought to the resident of Zola, Northern Nigeria, nearly all of whom were in a terrible condition, due to starvation.

He made a certain amount of progress at first, but did not improve as the others, and had constant diarrhea. The stools were watery and of a bilious color, no blood or mucus in the same. He was taken into the hospital, but died the same night, and on inspecting the stools passed during the night numerous reddish-yellow, translucent, gelatinous, oval bodies were found.

Post-mortem.—The lungs and heart were normal. Liver normal. The spleen small, hard, and black. The stomach contained some food, and on opening the small intestine the duodenum and upper part of the jejunum were found full of the oval bodies, none of them adherent, although they were alive. The mucous membrane was reddish, but no hemorrhages or petechiæ were apparent. The rest of the bowel was normal, a few of the oval bodies found loose in the large intestine. The kidneys were normal. The oval bodies have shrunken considerably, and are only about a third of the normal size.

These Pagans appear to be extremely fond of raw meat, and eat fowls raw.

As Shipley made a careful anatomical study of this parasite, and as our results differ in some respects from his, Shipley's account is here reproduced for comparison:

II. ANATOMY.

Alimentary canal.—There is no true sucker at the anterior end. The mouth is a simple aperture leading into a pharynx, the walls of which form an almost spherical bulb. The lumen is lined with chitin, and the bulb is separated from the general parenchyma of the body by a basement membrane. Between the basement membrane and the chitinous lining lies a loose tissue crossed by numerous muscle fibers, which mostly run in a radial direction, but a few run circularly.

At first the lumen of the pharynx is compressed from side to side, but after about 30–35 sections from the anterior end the lumen has become depressed from above downward, and just here are found two short dorsal and ventral valves projecting like tongues into the lumen, only directed backward. They are attached anteriorly and free posteriorly. Behind these valves the lumen becomes diamond-shaped, the long axis being the transverse one, and here the bulb is at its largest and occupies a good deal of the area within the body wall. Its wall is also now divided into an outer and inner layer by a well-marked layer of circular muscles. The inner layer consists largely of radiating muscle fibers. The whole bulb lies somewhat freely in the very loosely vacuolated parenchyma, which seems to form a space around it, transversed only by a few sparse threads of protoplasm.

As we pass into the posterior half of the bulb the diamond-shaped lumen becomes a slightly oval slit whose angles shortly afterwards are turned down, thus forming a crescentic-like space in cross section. At the hinder end of the bulb these turned-down corners are cut off from the central lumen and form two lateral diverticula, the pharyngeal pouches. The diverticula, although they have their origin in the turned-down corners of the lumen, soon come to lie dorso-lateral of the central channel, and this alteration in relative position is caused by the central channel passing toward the ventral surface of the body. The pharyngeal pouches consist of the same kind of loose vacuolated tissue as the bulb; they are very thick walled and with small lumina.

Behind the bulb the lumen of what may now be called the esophagus deepens, and in the region of the anterior border of the genital pore the central portion of the alimentary canal is no longer surrounded by the characteristic tissue of the bulb, though the two dorso-lateral diverticula, which still persist, are. The lumina of these diverticula then become slightly coiled so as to appear twice in one section, and then each of them fades out and disappears altogether. At about the level where the anterior third of the body joins the posterior two-thirds, the esophagus divides into the two lateral diverticula, and around the λ-shaped lumen at this point is a thick bulb or sheath of muscle fibers mostly circular in their arrangement, though some are radial. Around them is a layer of longitudinal muscles. The lateral diverticula now pass outward and begin to include between them the reproductive organs. Each diverticulum is flattened sideways and has a considerable dorso-ventral axis. They give off no secondary diverticula, though they are wavy or wrinkled, especially posteriorly, and here also they diminish in size, pass dorsally, and come to an end just about the level of the anterior lip of the great posterior sucker.

It does not seem possible to make out any cells lining any part of the gut. No epithelium is recognizable. The lumen is lined by a deeply-stained layer which looks like mucus, very thin in the pharynx, but quite thick in the intestinal diverticula. At the outer surface of this deeply-staining layer, darkly-stained structures, which may be nuclei, are here and there to be seen. The whole rests on a very definite basement membrane, and outside this in the region of the diverticula is a single layer of longitudinal muscles, the whole recalling in appearance the structureless lamella and the muscle tails of the ectoderm cells lying on it, in a hydra.

The excretory system.—The excretory pore lies in the middle line above the posterior sucker. It opens into a tube lined with cuticle directly continuous with that which clothes the body. This canal is pushed a little way out of the median line and lies, in the single specimen reduced to sections, a little to the left. Its walls soon thicken, and numerous darkly stained structures appear in its periphery; there may be nuclei or possibly sections through minute muscle fibers. Passing forward the canal enlarges and forms a spacious vesicle, which still lies over the sucker, spreading over its anterior end; from this vesicle, secondary canals pass up into the surrounding tissue. These, however, can not be traced farther in sections. The bladder or vesicle narrows again as we pass forward, and by the time the anterior edge of the sucker is reached it comes to lie between the hindermost ends of the diverticula of the alimentary canal. In front of this the main trunk seemed to divide into two, but beyond this they could not be traced.

The parenchyma. This packing or ground tissue consists of large cells usually diamond-shaped in section. They are evidently very soft, and have been pulled out into strand-like structures where the cuticle has been elevated. The cells contain a granular-looking protoplasm. The cells underlying the cuticle are much smaller than those of the parenchyma within; the details could not be made out, but amongst and between them are some obvious muscle fibers. Similar muscle fibers lie outside the gut-diverticula, and many such fibers surround the outer parts of the reproductive ducts.

The reproductive organs.—There is a genital papilla situated in the middle ventral line about the level where the anterior quarter joins the posterior three-quarters. On this open close together the canal of the cirrus and the metratrema, the vas deferens opening slightly in front of the latter. The whole papilla is but slightly projecting; its tissue is closer and firmer than the usual body tissue. The distal end of the cirrus canal is muscular for a short space, and seems to have glands opening into it, but it soon gives a bend and opens into a thin-walled vesicle on the ventral surface, the vesicula seminalis, which in the specimen that was cut into sections contained a mass of spermatozoa. The genital papilla is on a level with the lateral diverticula of the esophagus, but the vesicula seminalis lies beneath the muscular pharynx, just where the alimentary canal is beginning to split into two diverticula. Ventral to it lies the small vagina with muscular walls which, just behind the level of the opening of the cirrus canal into the vesicula seminalis, expands into the thin-walled uterus.

The vesicula seminalis opens into the vas deferens dorsally, and begins to pass backward as a slightly coiled, thick-walled duct. This is still packed with spermatozoa. The thick-walled duct suddenly passes into a thin-walled duct, which is closely coiled and still packed with spermatozoa. The junction of the two is at the level where the uterus begins to pass dorsally; it continues, however, to lie ventral to the coiled thin-walled portion of the vas deferens. The testes are double, and lie side by side, though one projects farther back then the other. They are ventral to the uterus, which for a short space lies between the glands and their ducts. The testes are closely adpressed to one another, and it is just possible that they unite at one point. They open straight into the thin-walled vas deferens. Each testis is deeply lobulated. The glands are packed with sperm morulas in various stages of development, their darkly stained nuclei giving the tissue a very characteristic appearance.

The metratrema or distal and modified end of the uterus opens close behind the vas deferens: it is a thick, muscular duct which passes backward for a short distance in a straight line. Just in front of the anterior border of the testes it enlarges into the uterus, and this begins to twist and loop, lying between the dorsally placed vas deferens and the ventrally placed testes. The uterus contains ova, but not in very great quantities; the eggs are incased in a shell and contain many deeply staining yolk granules, but little more can be made out. My measurements for an ovum, which looked unusually large, were 122μ by 80μ, but Conyngham gives 130μ by 75μ. Undoubtedly the eggs vary in size to a certain extent. The uterus coils a good deal over the testes, and at the posterior end of these glands its lumen enlarges, and it becomes filled with a glairy looking coagulum in which the ova lie embedded.

The ovary or germarium lies close behind the testes, and rather to the right of the body; it contains minute ova with large nuclei, closely packed together in some places and loosely in others. The whole, like the testes, is ensheathed in a connecting tissue casing. The oviduct leads from the anterior end and curves back above the ovary, it becomes almost immediately surrounded by the shell gland, and may here be called the ootype. Close behind the shell gland the ootype receives the opening of the vitelline duct and the inner end of Laurer's canal. The shell gland and the ovary come to an end at about the same level as the anterior edge of the posterior sucker. There is a well-marked canal of Laurer which passes almost directly dorsalward and opens in the dorsal middle line just in front of the posterior sucker.

The yolk glands are conspicuous, follicular structures, which take no stain, but remain a somewhat dirty-brown color, somewhat glistening. They extend forward as far as the reproductive pores, and they lie near the edge of the body, ventral to the right and left branches of the alimentary canal. The glands increase in number posteriorly, and in the region of the great sucker are very numerous. Their minute ductules fuse together and gradually unite into right and left ducts that open into the ootype, which is surrounded by the shell gland, and in which the egg is made up. Into the same space opens the duct of the yolk reservoir, which is a coiled receptacle, full of yolk, lying to the left and opposite the ovary.

III. SYSTEMATIC POSITION.

The trematode we have to do with has been described by Mr. H. F. Conyngham as a species of the genus *Amphistoma*, which he calls *Amphistoma watsoni*. Dr. F. Fischœder has recently pointed out that the name *Amphistoma* is in reality a synonym of the genus *Strigea*, but the original *Strigea* has since been described as *Holostomum macrocephalum*, and if *Strigea* is to be revived it must be for that form. Hence, Doctor Fischœder proposes to us the name *Paramphistomum* for what we have used to term *Amphistomum*, and the name *Paramphistomidæ* for the family to which they belong. Whether we follow the classification of Bronn's Thierreich or, as I propose to do, the later classification of Fischœder, it is impossible to class the new human parasite described above as an *Amphistomum*, because that species is characterized, amongst other things, by the absence of the lateral diverticula of the pharynx, which form so characteristic a feature of our species. This fact, however, could only be determined by cutting the animal into sections, and therefore escaped the notice of Mr. Conyngham.

Fischœder divides the *Paramphistomidæ* of the Mammalia into two subfamilies: (i) the *Paramphistomina* with the genera *Paramphistomum*, *Stephanopharynx*, and *Gastrothylax*, all these being devoid of pharyngeal side pouches, and (ii) the *Cladorchinæ* with the genera *Cladorchis*, *Chiorchis*, *Gastrodiscus*, *Homalogaster*, and *Balanorchis*. Of these genera *Cladorchis* is characterized by having the body not divided into anterior and posterior portions, by having the lateral edges rounded, by having the ventral surface slightly hollowed, and in all these respects our genus agrees with *Cladorchis*, and differs from the other members of the subfamily. I therefore place it in this genus.

CLADORCHIS WATSONI (Conyngham).

SYNONYM.—*Amphistomum watsoni* (Conyngham).

Length, 8–10 mm.; greatest breadth, 4–5 mm., tapering toward the anterior end to about 2.5 mm.; and depth about 4 mm.; color, when fresh, reddish-yellow, when preserved, a dirty brown; when fresh, translucent and gelatinous; the ventral surface transversely wrinkled, the aperture to the posterior sucker small, but the sucker itself big; no distinct sucker anteriorly but a well-marked pharyngeal bulb; the two pharyngeal pouches project beyond the outer limit of the bulb; circular sphincter round the esophagus just where it forks; distinct genital papilla, testis lobed, divided into two, side by side, anterior to ovary; Laurer's canal straight, opening anteriorly to excretory vesicle in middle line above the posterior sucker; the latter is very large and vaulted; the ova measure from 122–130μ by 75–80μ.

HABITAT.—*Homo sapiens*, a West African negro, jejunum and duodenum, very few in the large intestines.

So far as we are aware, all other reference to this species are based upon the foregoing papers.

EXTERNAL CHARACTERS.

SIZE.—Conyngham (1904) states that the worms measure 8 mm. in length and 5 mm. in greatest breadth, tapering gently cephalad to 2.5 mm. in breadth, and 4 mm. in greatest thickness.

Shipley (1905) gives the length as from 8 to 10 mm., greatest breadth 4 to 5 mm., tapering toward the oral extremity to about 2.5 mm., and 4 mm. in dorso-ventral diameter.

COLOR.—In Watson's clinical notes quoted by Conyngham (1904) the fresh specimens are described as reddish-yellow, translucent, gelatinous bodies. Conyngham states that after fixing and in the preserved state they become a dark slate color. Shipley (1905) gives the color as reddish-yellow, translucent, and gelatinous in the fresh state and a dirty brown when preserved.

FORM.—Conyngham describes them as pear shaped (figs. 175, 176), flattened ventrally and slightly posteriorly at the margin of the posterior sucker, but very much shrunken from the action of the preservative.

SURFACE.—Conyngham states that the cuticle is marked by transverse ridges which are more coarse and better defined on the ventral surface. The anterior sucker is described as being retracted in most of the specimens and as lying at the bottom of a ventro-terminal sulcus. The venter is described as flattened, surrounded by an elevated ridge and as bulging posteriorly.

Genital pore.—The genital pore is given as being 2 mm. from the anterior sucker, presumably in the ventro-median line, or about one-fourth of the length of the parasite from the anterior end and is stated to be rather prominent. Shipley (1905) states that there is no true oral sucker, and that the venter is transversely wrinkled. Shipley mentions a genital papilla as situated in the midventral line about the level where the anterior fourth joins the posterior three-fourths.

Acetabulum.—Conyngham states that the "posterior sucker is very large, its cavity measuring over 1 mm. across; it is subterminal and ventral." Shipley describes the posterior sucker as big, but with a small aperture.

We find that the rim of the acetabulum projects considerably beyond the embrace of the body parenchyma in a manner very similar to that which obtains in *Ps. stanleyii* and, as in the latter, it forms a ring around the aperture, being encircled by a deep, narrow groove (figs. 175, 189), which marks it from the general surface.

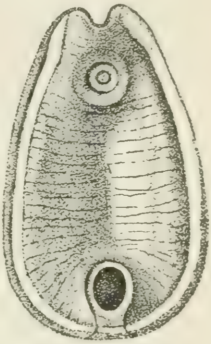

FIG. 175.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The oral extremity of the worm is marked by a dorso-ventrally directed groove-like depression which encroaches slightly on the ventral surface. By Conyngham this is described as a ventro-terminal sulcus and this is pictured, though not mentioned, by Shipley (fig. 175). The surface of this depression is beset by digitate papillæ. It leads by an irregularly circular aperture about 165μ in diameter directly into the oral sucker. The latter (figs. 178, 179) is a large organ; in length it equals about one-fifth of the total body length. Its maximum transverse and dorso-ventral diameters are at about its equator, and measured from sections are 1.2 mm. and 1.1 mm., respectively. These diameters decrease in the direction of both poles, but more particularly toward the oral pole, which is bluntly pointed. The decrease in these diameters, in the direction of the caudal pole or base, is progressive though slight. A little above the level of the base the decrease in the transverse diameter ceases; soon this diameter begins to expand, this expansion being due to

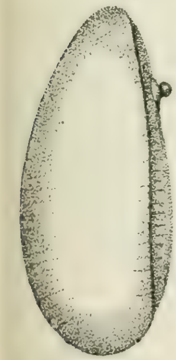

FIG. 176.

the extension at first laterad and then dorso-laterad in the form of pouches of the sucker from the region of its caudo-lateral aspect. The ventro-dorsal diameter, however, continues progressively to decrease, the base of the sucker viewed in sagittal plane being rounded, tilted somewhat ventrad and giving origin to the esophagus.

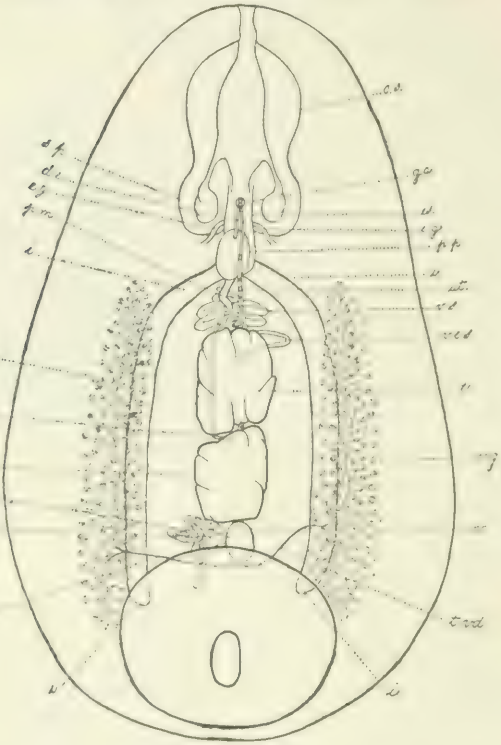

Fig. 177.

The pouches are irregularly globular in form and as they extend latero-caudad come to lie close to the dorso-lateral aspects of the first portion of the esophagus. The caudal third of the sucker and its pouch-like prolongations are in a well-marked perisuctorial space (fig. 181), in which they are retained in position by mesenterium-like strands extending from the parenchyma particularly to the dorsal and ventral aspects of the sucker. The structure of the suctorial wall differs somewhat at different levels. At the oral pole the suctorial wall consists of parenchyma-like cells with some radial, circular, and longitudinal muscular fibers arranged beneath the cuticular lining of the lumen. Farther caudad, however, these muscular fibers increase in number and except for the radial bundles are massed into a well-defined inner zone as contrasted to an outer zone of the parenchyma-like cell structure. Just above the level of origin of the pouches the inner muscular zone forms the greater portion of the thickness of the wall, the cell structure of the outer zone at the same time becoming greatly condensed. The structure of the pouch walls shows a similar inner relatively narrow muscular zone in which the circular fibers are most prominent, and an outer parenchyma-like zone.

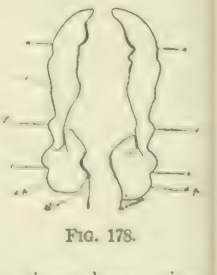

Fig. 178.

The lumen of the sucker, in a general way, is a dorso-ventrally narrow, but transversely a relatively broad space. Caudo-laterally it extends into the caudo-lateral prolongations or suctorial pouches of the sucker. Besides variations in the dorso-ventral diameter of the lumen at different levels, naturally to be expected from irregularities in the degree of contraction at the time of fixing, there are differences due to peculiarities in form of the suctorial wall itself. Beginning at the oral aperture of the sucker the lumen for some distance caudad maintains a fairly uniform dorso-ventral diameter, then rather abruptly this becomes decidedly increased. This increase is due to a retraction in the dorsal and in the ventral suctorial wall so as to form what Shipley describes as "dorsal and ventral valves projecting like tongues into the lumen, only directed backward" (fig. 179). These transverse projecting tongues or ridges are not continuous laterally; the interval thus left increases to a corresponding degree the dorso-ventral diameter of the lumen at its lateral angles; the form in transverse section of the lumen at the level where these ridges are formed suggests to a slight extent the letter H (fig. 180).

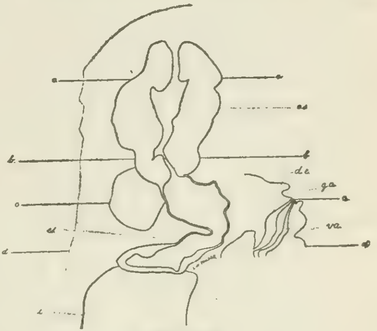

FIG. 179.

Almost at once, however, the dorso-ventrally expanded lumen resulting from the retraction of the dorsal and the ventral suctorial walls, above described, begins to contract and continues progressively to decrease to the level of origin of the pouches. In this region the lumen becomes very abruptly greatly narrowed dorso-ventrally by the projection upward into the lumen from its dorsal wall of a transverse tongue-like ridge recalling a similar structure in *Ps. stanleyii* (fig. 179). In transverse sections the first portion of the suctorial lumen is a transverse slit, the second portion is at first fusiform or diamond-shaped in outline, eventually becoming crescentic with the concavity of the crescent ventrad (fig. 181). By the projection

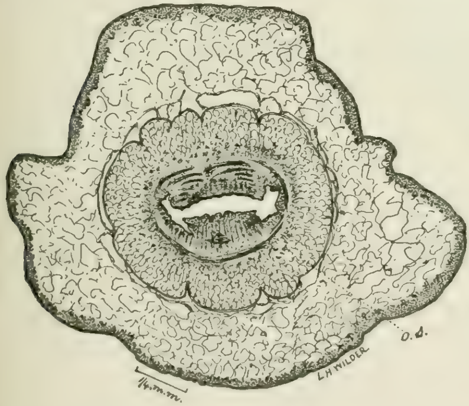

FIG. 180.

upward of the tongue-like transverse ridge the horns of the crescentic lumen become partly separated from the body, so that at this level the lumen, as in *Ps. stanleyii*, somewhat suggests the letter H. The terminal portion of the suctorial lumen also appears as a transverse slit in section.

The lumen of the sucker and that of its pouches is lined with a cuticle-like layer; in the first portion of the sucker the cuticle is beset with conical papillæ of moderate size.

From its point of origin the esophagus passes at first ventro-caudad, then at about its equator it bends abruptly and sharply dorsad with a tilt caudad. Viewed ventrally the esophagus is apparently much shorter than the sucker, but in sagittal plane it is at once seen that it slightly exceeds the length of the latter. The esophageal wall is muscular throughout, but in the caudal half the muscular layer is particularly well developed, attaining a maximum thickness of about 67μ. Because of the obliquity of this portion of the esophagus certain of the transverse sections cut its wall almost tangentially, and consequently the observer is readily misled into interpreting such a section as indicating an enormously thick muscular wall (fig. 183). Viewed ventrally, therefore, this portion of the esophagus would have somewhat the appearance of a muscular bulb, such as Shipley describes and pictures.

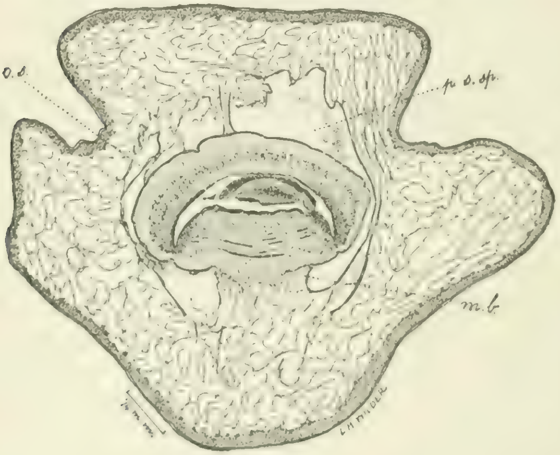

Fig. 181.

The first half of the esophagus is dilated in the dorso-ventral diameter, but compressed from side to side. The esophageal lumen is lined throughout with a rather thick cuticular layer.

The intestines spring from the lateral aspects of the caudal portion of the esophagus. At first they arch caudo-laterad, they then pass directly caudad in relation to, though at some distance from, the dorso-lateral aspect of the body. They terminate by cecal extremities slightly caudad of the junction of the fourth with the caudal fifth of the body length, or slightly caudad of the plane of the cephalic margin of the acetabulum, the right tube extending slightly farther caudad than the left. In transverse section the ceca appear compressed from side to side with proportionately a greatly elongated dorso-ventral diameter, the former bearing a relation of 1 to about 5 of the latter.

Genital system.—With the exception of the vitellaria and the copulatory apparatus the genital organs are situated in the intercecal area.

Male organs.—The testes are in the axial region of the body, though somewhat nearer the venter than the dorsum; the superior testis is in the equatorial zone of the worm, occupying in this region about one-seventh of the body length; the inferior or caudal testis also occupies about one-seventh of the body length in a zone contiguous to and immediately caudad of that of the superior testis. Both testes are deeply indented by fissures and sulci in such a manner as readily to lead to the erroneous interpretation that the testes are side by side in close apposition, particularly as the caudal aspect of the superior and the cephalic aspect of the inferior testis are in close apposition and their contiguous lobes marked off by their fissures and sulci overlap slightly, and consequently portions of both testes appear in certain of the transverse sections (fig. 185).

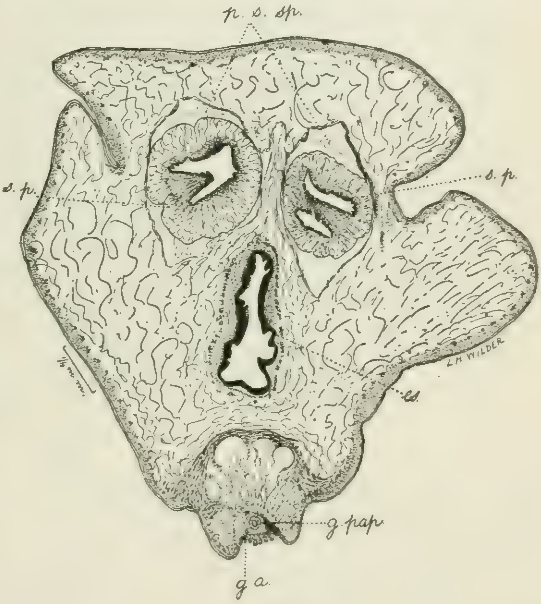

Fig. 182.

A vas efferens springs from the dorso-cephalic aspect of each testis (fig. 184); then it passes cephalo-dorsad, that from the superior testis tending to the left and that from the inferior to the right of the median sagittal plane. At about the level of the cephalic aspect of the superior testis the left vas efferens approaches close to the mesial aspect of the left intestine; it then curves inward as it courses cephalo-dorsad and very soon enters the complex of the coils formed by the vas deferens amongst which it can not be followed. The right vas, as already stated, passes cephalo-dorsad and to the right immediately after its origin from the caudal testis. It skirts the caudal aspect of the superior testis, then after reaching the right caudo-dorsal aspect of this testis it bends and proceeds almost directly cephalad in close relation to the right dorso-lateral aspect of the superior testis until it reaches a level a little short of that at which the left vas efferens originates, when it begins to tilt dorsad, at the same time becoming considerably distended with spermatozoa (fig. 184). Shortly beyond this point it begins to wind and enters the coil-complex of the vas deferens, beyond which point it is impossible to trace it satisfactorily. The two vasa efferentia presumably enter into the formation of the vas deferens and it would appear, though this can not be made out satisfactorily in this series of sections, as if each, before their union, became considerably distended and coiled, their coils being indistinguishable from those of the first portion of the vas deferens.

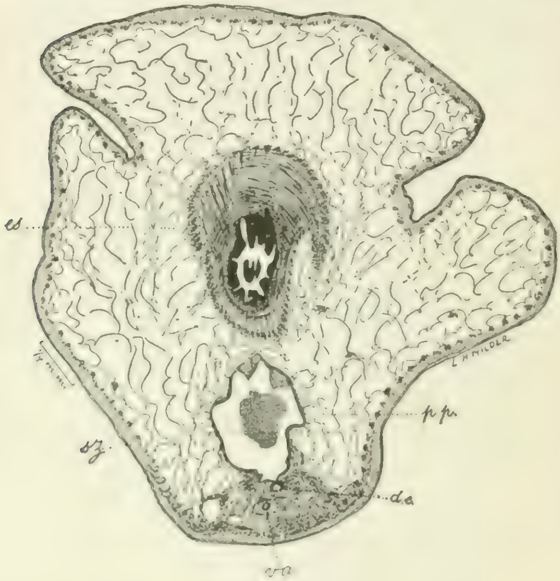

FIG. 183.

The vas deferens presents at first a thin-walled intricately coiled dilated portion or vesicula seminalis. These coils are in the intercecal space, elongated from venter to dorsum and dorsum to venter and winding cephalad; they are succeeded by a muscular-walled segment or pars musculosa, the wall of which, measured at a favorable point, was about 60μ thick. This portion is relatively short, uncoiled, though making about one spiral turn in its somewhat sinuous course ventro-cephalad. In its turn, at about the level of the esophageal fork, this is abruptly succeeded by a short greatly dilated portion with muscular walls intermediate in thickness between those of the vesicula and pars musculosa. This portion, which Shipley interpreted as the vesicula seminalis and which corresponds to the vesicula seminalis interna of the forms with a cirrus pouch, appears homologous with the type of pars prostatica of *Homalogaster philippinensis* and that of *Ps. stanleyii*, more particularly the latter, in which the prostatic cells are few, while in this (*Watsonius watsoni*) species no prostatic cells at all can be distinguished. It is abruptly succeeded by a thin walled duct of a much smaller caliber which with the terminal portion of the uterus close to its ventral aspect at once plunges into a sharply delimited muscular mesh as it proceeds cephalo-ventrad. This is relatively long and eventually opens at the vertex of a genital papilla by a small pore separate from and immediately cephalad of the opening of the metraterm. This terminal portion of the vas deferens is homologous with the ductus ejaculatorius. It may be well to describe at this point what may be designated as the copulatory apparatus.

In the median line of the ventral surface at a point about one-fourth the length of the worm from its oral margin is a well-marked ring-like elevation or bulging which encircles a second more sharply defined truncated cone-like bulging measuring about 225 μ from base to vertex, about 600 μ in transverse diameter at the base, and about 375 μ at the vertex. The vertex of this second cone-like projection is depressed or crateriform and may perhaps be regarded as the genital atrium, from the dorsal wall of which the genital papilla projects. These structures are well shown in surface view in figure 175 and in transverse section in figure 182. The crateriform depression into which the genital papilla projects is beset by numerous quite small papillæ. The form of the genital papilla can not be made out satisfactorily;

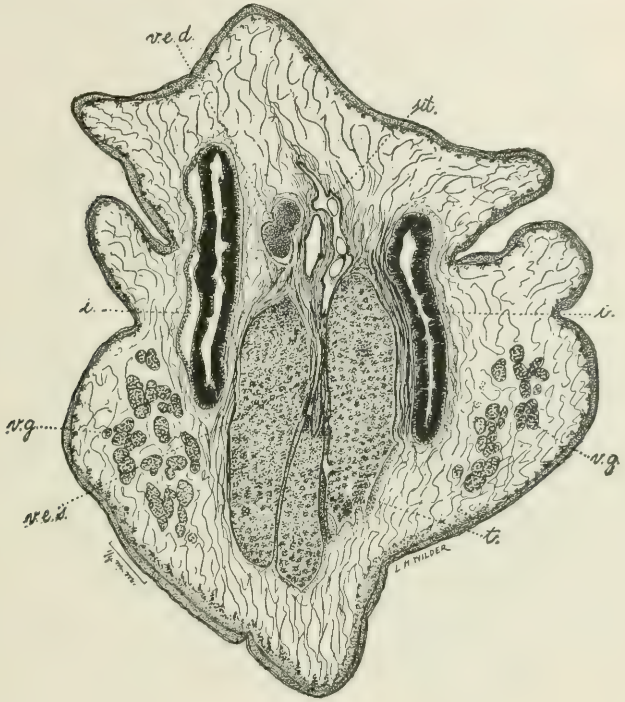

FIG. 184

one gains the impression that it is a low, broad, rounded elevation. In sections it may be seen that the internal structure of the genital bulging is made up of a muscular mesh which is sharply delimited from the body parenchyma by a well-defined curved (with convexity dorsad) muscular layer of transverse, radiating, and vertical bundles. It is this curved limiting layer (somewhat suggestive of a cirrus pouch) that, as already mentioned, is pierced by the ductus ejaculatorius and the terminal portion of the uterus or metraterm. The structure of this copulatory apparatus suggests the probability that it may be collapsible or retractible. The genital papilla is just caudad of the level of the base of the sucker (or origin of the esophagus).

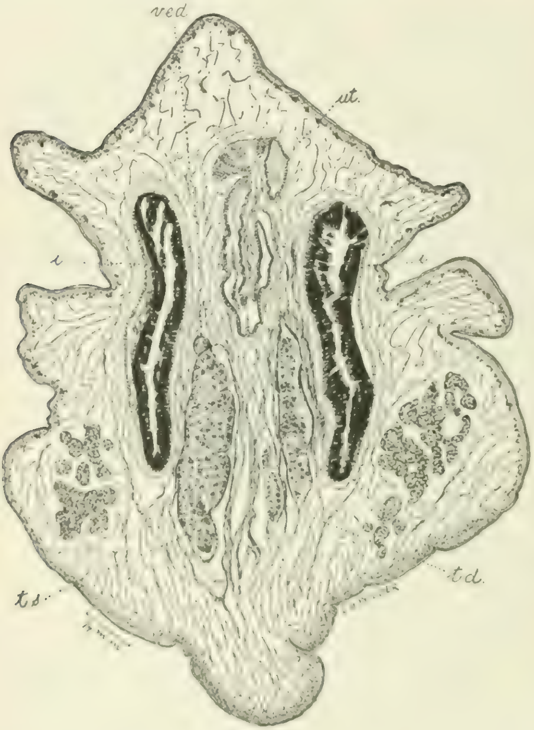

FIG. 185.

Female organs.—The ovary is in the axial region of the body in the intercecal area a little nearer the left than the right intestine, caudo-dorsad of the caudal testis and dorso-cephalad of the acetabulum and in a zone directly caudad of and slightly overlapping the zone of the caudal testis (fig. 186) superiorly and to a slight degree overlapping the acetabular zone inferiorly (caudally). The ovary is dorso-ventrally elongate, measuring about 0.66 mm. in this and about 0.25 mm. in the transverse diameter. From its dorsal-cephalic aspect (fig. 186) the oviduct takes origin. This passes directly dorsad for about 150μ of its length and then bends caudad, almost immediately penetrating the cephalic aspect of the shell gland, at the same time giving off Laurer's canal. The latter proceeds dorsad (fig. 187) with but a slight inclination caudad and reaches the dorsum in about the median line or only slightly if at all to the right of it, and at a point in a plane marking the caudal limit of the ovarian zone and therefore only slightly caudad of that of the superior limit of the acetabular zone (or superior margin of the acetabulum) (fig. 188).

The shell gland is placed directly dorsad of the ovary; the caudal limit of its zone is the same as that of the ovarium, though because the vertical diameter of the shell gland is slightly less than that of the latter the zones of the two glands are not quite coextensive, the upper (cephalic) limit of the shell gland being slightly below (caudad) of that of the ovary. As already mentioned the oviduct penetrates the cephalic aspect of the shell gland, in the substance of which it unites with the common vitello-duct. The duct resulting from this union at once forms a fusiform dilatation, the ootype, which passes ventrad with a slight obliquity to the right and caudad in the major axis of the shell gland. The continuation of the ootype becomes the uterus which emerges from the ventral pole of the gland (fig. 187). After emerging, the uterus passes vento-dextrad into the field between the ovary and the right intestine, but doubles back before it has quite reached the frontal plane of the ventral margin of this intestine, and thus completes a loop directed ventrad. On reaching a point to the right of the dorsal pole of the shell gland it dips caudad, the loop thus formed

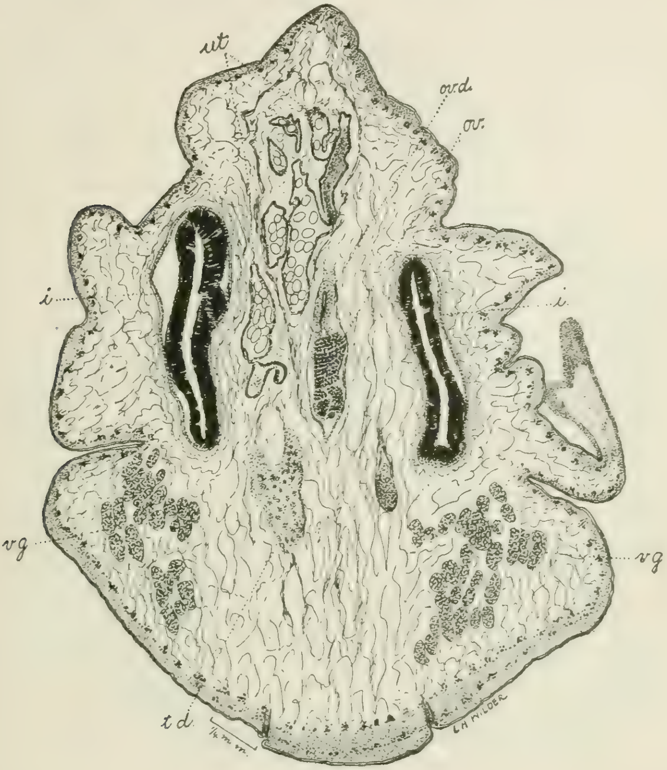

FIG. 186.

coming into close relation to the right aspect of the dome of the excretory vesicle. After forming this loop the uterus continues dorsad until it reaches the field to the right of the line of Laurer's canal and dorsomediad of the right intestinal cecum, where it forms some coils and begins its ascent cephalad, forming dorso-ventral loops, at first in the field between the right intestine on the one side and the ovary and shell gland on the other; later these loops are in the median line in the interceecal area between the testes and the dorsum. The uterus winds its way cephalad in this field, dorsad of the testes, until it reaches the level of the caudal aspect of the vas deferens. Here it tends ventrad to gain the ventral aspect of the vas deferens, arching across the cephalic aspect of the superior testis. At the same time it ceases to form coils, proceeding in a sinuous but direct course cephaloventrad. From the level of the esophageal fork it is continued as the metraterm, and, as already mentioned, this pierces the muscular mesh of the copulatory apparatus to open immediately caudad of the ductus ejaculatorius at the vertex of the genital papilla.

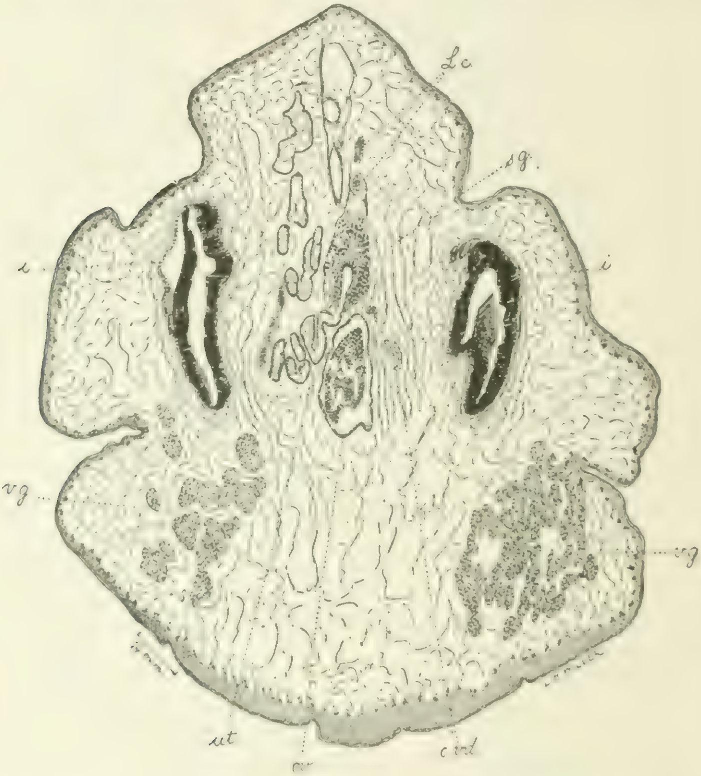

FIG. 187.

The first loop formed by the uterus after its emergence contains a considerable number of vitelline cells, suggesting the idea of a yolk reservoir. It is probably on this account that Shipley was led into interpreting this as a separate structure, to which he applied the name "yolk reservoir." In the remaining loops a considerable number of eggs were noted. Here and there in the coils dorsad of the testes there are masses of spermatozoa in which some of the eggs are embedded.

The vitellogene glands, consisting of a moderate number of loosely aggregated, well-developed follicles, are situated in the fields between the ceca and the ventro-lateral aspect of the body; that is, ventrad and ventro-laterad of the ceca and ventro-laterad of the upper (cephalic) portion of the acetabulum. Longitudinally they extend from about the level of the esophageal fork to or slightly caudad of the level of the upper margin of the acetabular aperture. The gland of the left side is a little shorter than that of the right. A duct of considerable caliber, distended with yolk cells, leaves the gland of each side—that of the left at a point slightly cephalad of the superior margin of the ovary; that of the right at about the level of the ootype. These ducts, the transverse vitello-ducts, pass dorso-mediad and more or less caudad ventrally of the intestinal ceca to unite close to the

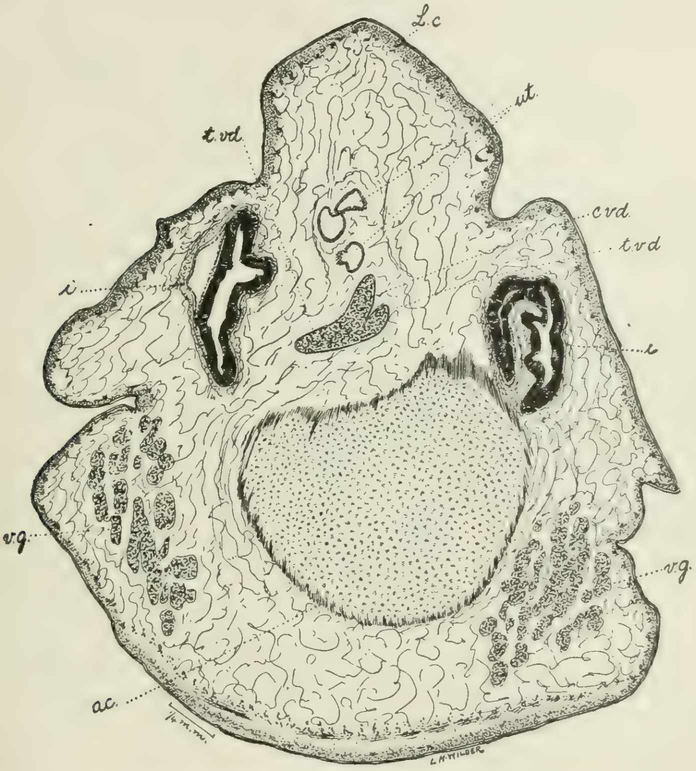

FIG. 188.

caudal aspect of the shell gland. From their point of union, which is not dilated into a reservoir, a common vitello-duct arises and passes very obliquely dorso-cephalad and almost at once penetrates the shell gland. The common, like the transverse ducts, is distended with yolk cells and at first is of about the same caliber as the latter, but as it enters the substance of the shell gland its caliber becomes rapidly reduced in diameter. As has already been stated, it joins with the oviduct to form the ootype.

Excretory system.—The excretory vesicle is in the caudal portion of the body, dorso-cephalad of the acetabulum. It is relatively small; its dome extends a short distance cephalad into the caudal portion of the interceca space, attaining the level at which the vitello-ducts unite; caudad it extends to about the level of the upper margin of the acetabular aperture. Here it gives off a thick-walled duct which passes dorso-caudad to open on the dorsum, apparently slightly to the left of the median line, at a point in a plane slightly cephalad of that of the owermargin of the acetabular aperture, and therefore at some considerable distance caudad of the pore of Laurer's canal. The excretory duct is lined with a cuticular layer. Excretory canals are seen to enter the excretory vesicle, but they can not be satisfactorily traced.

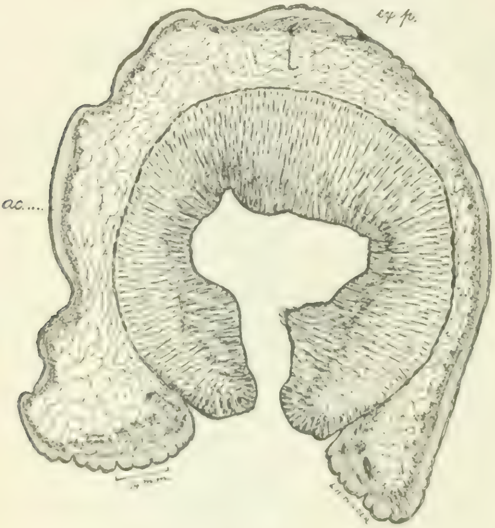

Fig. 180

ILLUSTRATIONS.

Fig. 175.—Ventral view, × about 4. (After Shipley, 1905, fig. 1.)

Fig. 176.—Profile view, × about 4. (After Shipley, 1905, fig. 4.)

Fig. 177.—Ventral projection (diagrammatic) to show internal anatomy: *d. e.*, ductus ejaculatorius; *es.*, esophagus; *e. g.*, esophageal ganglion; *g. a.*, genital atrium, with openings of ductus ejaculatorius (upper pore) and metraterm (lower pore); *i.*, intestinal ceca; *o. s.*, oral sucker; *ov.*, ovary; *p. m.*, pars musculosa; *p. p.*, (?) pars prostatica; *s. g.*, shell gland (dorsally of ovary); *s. p.*, suctorial pouch; *t.*, testes; *t. vd.*, transverse vitello-ducts; *ut.*, uterus; *v. e. d.*, right vas efferens; *v. e. s.*, left vas efferens; *v. g.*, vitellaria; *v. s.*, vesicula seminalis. Enlarged. Original.

Fig. 178.—Ventral projection of oral sucker (*o. s.*), suctorial pouches (*s. p.*), and portion of esophagus (*es.*). *a–a*, *b–b*, *c–c*, planes of section. Enlarged. Slightly diagrammatic. Original.

Fig. 179.—Profile projection of oral sucker (*o. s.*), suctorial pouch of left side (*s. p.*), and esophagus (*es.*). Shows also the position of the genital atrium (*g. a.*). *a–a*, *b–b*, *c–c*, *d–d*, planes of section. Slightly diagrammatic. Enlarged. Original.

Fig. 180.—Transverse section through plane *a–a* figs. 178 and 179. Shows oral sucker (*o. s.*); H-formed lumen of oral sucker (with papillæ) at this level. Enlarged. Original.

Fig. 181.—Transverse section through plane *b–b* figs. 178 and 179. Shows crescentic lumen of oral sucker (*o. s.*), with transverse tongue-like ridge projecting upward into it. Shows also perisuctorial space (*p. s. sp.*) with ventral mesenterial band (*m. b.*). Enlarged. Original.

Fig. 182.—Transverse section through plane *c–c* figs. 178 and 179. Shows genital bulging with genital atrium (*g. a.*) beset with papillæ; the genital papilla (*g. pap.*) with opening of the ductus ejaculatorius; the limiting muscular layer of the copulatory apparatus; the esophagus (*es.*), suctorial pouches (*s. p.*), and extensions of the perisuctorial space (*p. s. sp.*) inclosing the pouches. Enlarged. Original.

Fig. 183.—Transverse section through plane *d–d* fig. 179. Shows metraterm (*va.*), ductus ejaculatorius (*d. e.*) just after its departure from the (?) pars prostatica (*p. p.*), which contains a mass of spermatozoa (*sz.*), and the esophagus (*es.*) with its dorsal wall cut tangentially, giving the impression of great thickness. Enlarged. Original.

Fig. 184.—Transverse section at level of origin of left vas efferens (*v. e. s.*). Shows terminal portion of right vas efferens (*v. e. d.*) distended with spermatozoa, the superior testis (*t.*), uterus (*ut.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 185.—Transverse section through overlapping portions of the superior testis (*t. s.*) and inferior testis (*t. d.*). Also shows uterus (*ut.*), intestine (*i.*), vitellaria (*v. g.*), and right vas efferens (*v. e. d.*). Enlarged. Original.

Fig. 186.—Transverse section through caudal extremity of inferior testis (*t. d.*). Shows ovary (*ov.*) with oviduct (*ov. d.*), uterus (*ut.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 187.—Transverse section immediately above level of superior margin of acetabulum. Shows ovary (*ov.*), shell gland (*s. g.*) with ootype and emerging uterus (*ut.*), Laurer's canal (*L. c.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

Fig. 188.—Transverse section at level of the pore of Laurer's canal (*L. c.*). Shows acetabulum (*ac.*), formation of common vitello-duct (*c. vd.*) by the union of the transverse vitello-ducts (*t. vd.*), loop of uterus (*ut.*), intestines (*i.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 189.—Transverse section at level of excretory pore (*ex. p.*). Shows acetabulum (*ac.*) with projecting rim of aperture. Enlarged. Original.

PSEUDOCLADORCHIS Daday, 1907.

GENERIC DIAGNOSIS.[a]—*Cladorchiinæ* (p. 169): Body rather cylindrical, venter rather convex, dorsum convex, cephalic end rather attenuate, caudal end rounded, sides rounded. Ventral pouch absent. Acetabulum ventro-subterminal, large; aperture circular, medium, directed slightly ventrad. Genital pore without sucker. Excretory pore postvesicular, in equatorial zone, caudad of pore of Laurer's canal. Oral sucker with 2 sphincters, one anterior, the other esophageal, and with paired not very well-developed evaginations; esophagus springs from caudal end of sucker and is without muscular thickening; ceca straight or slightly wavy, rather long, end postequatorial, preacetabular.

Male organs: Testes 2, much smaller than acetabulum, elongate, lobate, fields separate or abut, zones overlap or nearly coincide, preovarial, quite removed from acetabulum, never in caudal third; cirrus pouch present.

Female organs: Ovary and shell gland posttesticular; vitellaria never pretesticular, are close to ceca in cecal to postcecal zones, chiefly posttesticular, "branched, tree like;" uterus chiefly in intercecal field, with tendency to transverse slings, ventral of cecal plane; Laurer's canal chiefly prevesicular, does not cross excretory canal or vesicle.

TYPE SPECIES.—*P. cylindricus* (Dies., 1836).

PFENDERIUS,[b] new genus.

GENERIC DIAGNOSIS.—*Cladorchiinæ* (p. 169): Body rather conical, dorsum convex, venter slightly convex, cephalic end attenuates gradually but considerably, caudal end slightly, sides rounded. Ventral pouch absent. Acetabulum terminal, with projecting margins, relatively large, its shallow cavity provided with prominent papillæ, aperture large. Genital pore without sucker. Excretory pore in vesicular zone, in acetabular zone, postcecal, caudad of pore of Laurer's canal. Oral sucker with one (anterior) sphincter, and with a pair of well-developed evaginations; esophagus springs from ventral aspect of base of sucker and is without muscular swelling; ceca wavy, long, end postequatorial in acetabular zone.

Male organs: Testes 2, very much smaller than acetabulum, lobate, fields separate, zones coincide, considerably removed from acetabulum, preovarial, equatorial; cirrus pouch present.

Female glands: Ovary and shell gland distinctly and considerably posttesticular; vitellaria in cecal zone, from bifurcation to end of ceca, with sparsely scattered small follicles; uterus intercecal, with marked tendency to dorso-ventral slings; Laurer's canal entirely preexcretory, does not cross excretory canal or vesicle.

Eggs: Operculated, rather numerous.

TYPE SPECIES.—*Pfenderius papillatus* (Cobbold, 1882) as represented by U. S. N. M. 2554.

HABITAT.—Colon of elephants, India.

PFENDERIUS PAPILLATUS (Cobbold, 1882) Stiles & Goldberger, 1910.

[Figs. 190 to 202.]

1882 *Amphist. papillatum* Cobbold, 1882a, 240-242, figs. 10, pl. 21, fig. 11 (in *Elephas indicus*).—Braun, 1892a, 580, 663; 1893a, 874, 905; 1893d, 466.—Fischoeder, 1902a, 49 (in *Elephas indicus;* India).—Sonsino, 1895, 184, 187, figs. 4–5.

SPECIFIC DIAGNOSIS.—*Pfenderius* (p. 232): Body 4.5 mm. to 5.5 mm. long, 2.5 to 2.75 mm. broad, 1.7 mm. thick; pearl tint or opaque olive green in color (alcohol specimen);

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[a] Based on Daday, 1907.

[b] Dedicated to Dr. Charles A. Pfender, in recognition of his work on the Index-Catalogue of Medical and Veterinary Zoology.

rather conical, but bent slightly ventrad, greatest diameter between third and fourth fourth of body, attenuating gradually and considerably cephalad, slightly caudad; dorsum convex longitudinally and transversely, venter slightly convex from side to side, straight to slightly convex longitudinally; lateral margins curved both longitudinally and in transverse plane; transverse section of body transversely elliptical to circular. Genital pore in esophageal zone about one-fourth of body length from oral margin. Acetabulum terminal, with projecting margin, about 1.7 mm. in transverse, 1.4 mm. in dorso-ventral diameter, aperture directed slightly ventrad, 1.28 by 1.2 mm., cavity shallow, surface with prominent papillæ which attain 90μ long by 60μ broad at base. Mouth terminal at bluntly pointed cephalic extremity, with small papillæ; oral sucker with 2 caudal lateral bulbs, and with a well-defined sphincter about 120 to 140μ from oral margin; perisuctorial space very narrow; esophagus markedly curved dorsad, convexity ventro-caudad; ceca wavy, extend to acetabular zone, then curve slightly cephalad and terminate. Excretory pore about dorso-median, slightly caudad of preacetabular transverse plane; excretory duct almost transverse; excretory vesicle well developed, dorsal of cephalic half of acetabulum.

Male organs: Testes equatorial, ventral of ceca, fields separate, zones nearly coincide; irregularly globular, 0.4 mm.; vasa efferentia rather short, run dorso-cephalad, unite about on pretesticular plane; vesicula seminalis coiled; cirrus pouch pyriform, large, 0.44 mm. long, greatest diameter 0.32 to 0.34. mm., muscular wall 0.12 mm.; ductus hermaphroditicus present.

Female organs: Ovary posttesticular, intercecal, preacetabular, nearly or quite median, at junction of equatorial and caudal third of body; shell gland caudo-lateral of ovary; vitellaria with sparsely scattered small follicles, external, ventral, and to some extent dorsal of ceca, in cecal zone from bifurcation to end of ceca; uterus forms dilated dorso-ventral slings in suctorial field, to near cirrus pouch, then runs more directly cephalo-ventrad to ductus hermaphroditicus; Laurer's canal runs dorsally in curve (convexity caudad) in zone of shell gland, to pore slightly dextrad of median line, about 0.5 mm. cephalad of excretory pore.

Eggs: Rather numerous, elliptical, about 150 by 70μ, operculated at one pole and bearing short knob at the opposite pole.

TYPE.—Unknown. Cotypes U.S.N.M. 1721, 2554, 5777.

HABITAT.—Colon of elephant (*Elephas indicus;* India).

SOURCE OF MATERIAL.—The material consists of 7 specimens which were found in 3 bottles, as follows: 5 specimens in bottle No. 5777, 1 in bottle No. 2554, and 1 in bottle No. 1721.

The labels in these bottles bear the following data: "Name *Amphistoma papillatum;* Host *Elephas indicus;* Locality India; Determined by T. S. Cobbold; Date 1882; Presented by T. S. Cobbold; Date, 22, XII, 1882." Our material therefore represents Cobbold's original specimens.

HISTORICAL REVIEW.—Cobbold (1882a, 224, 240–242, fig. 8, pl. 24, fig. 11) originally described this species with the following diagnosis:

> Body of a bright pink color, smooth, conical, bluntly pointed in front, broadly rounded off behind, with fine and regularly disposed transverse rugæ forming distinct rings in the region of the head. Caudal sucker subterminal, very large, its cup being armed with numerous large fungiform papillæ, closely set, and regularly disposed over the entire surface of the concavity. Reproductive papillæ placed well forward. Length, one-sixth to one-fourth of an inch. Breadth, one-eighth to one-seventh of an inch. Hab. Large intestine of *Elephas indicus.*

Cobbold's figure 11, drawn from a fresh specimen, shows certain anatomical details. The outline is rather different from that of our specimens. The position of the genital pore agrees fairly well, though not exactly, with its position in our material. The acetabulum and its aperture are relatively large. No indication of suctorial evaginations is given; the esophagus is short; the ceca are long, not wavy, and end in the acetabular zone. The testes are figured as relatively much larger than those in our specimens, and as having zones which overlap slightly, fields which coincide. This latter condition does not agree with our material, which distinctly shows coinciding testicular zones and separate fields.

FIG. 190.

Braun (1892a, 580, 663; 1893a, 874, 905) refers to the papillæ in the acetabulum, to the short esophagus, and mentions the worm as a parasite in the colon of *Elephas indicus*. His later reference (1893d, 466) merely cites the worm as a parasite of the elephant.

Sonsino (1895, 184, 187 (6, 9), figs. 4-5) figures *Amphist. papillatum* with an outline so very distinct from Cobbold's figure that a question might arise as to whether he is dealing with the same species.

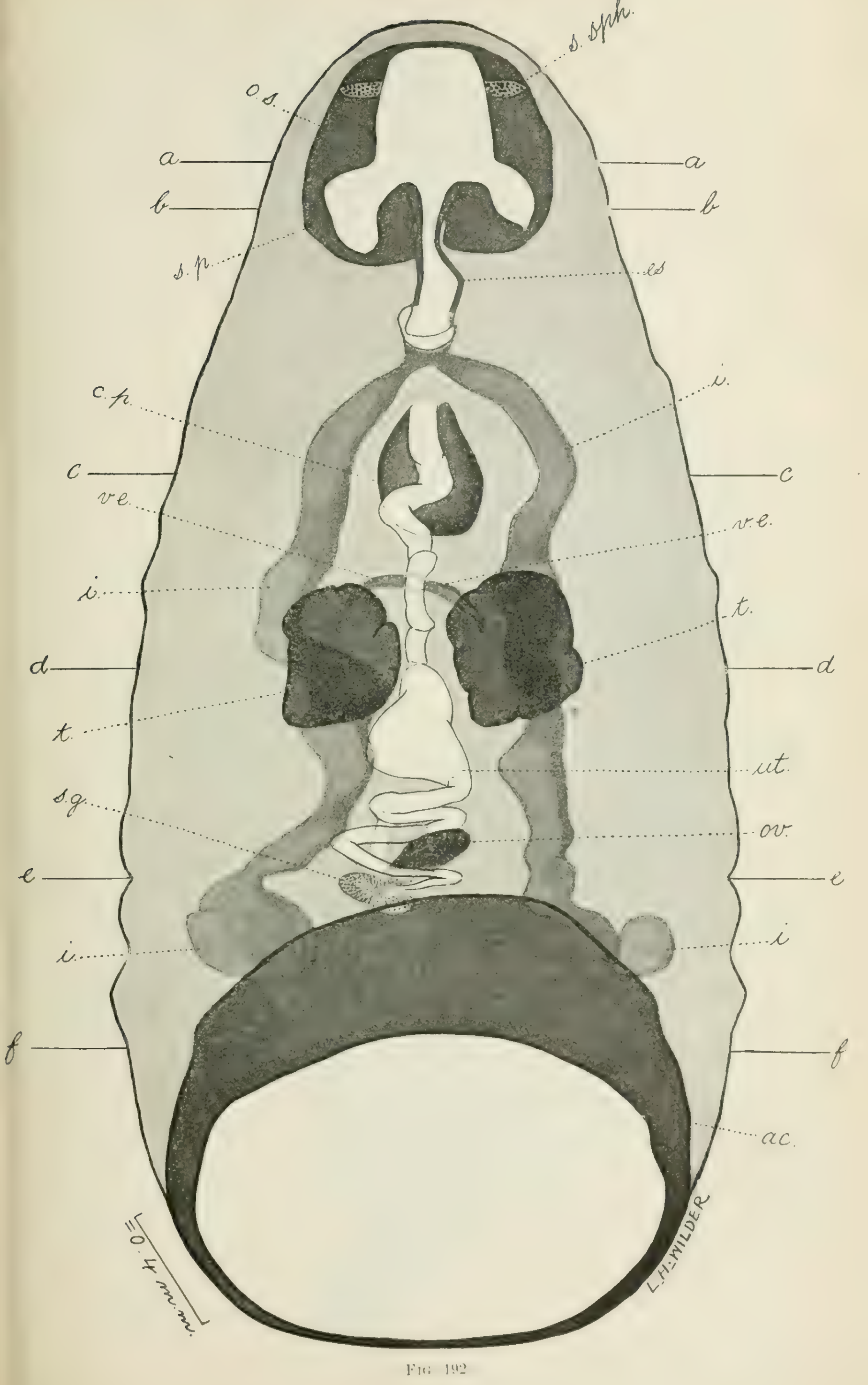
Fischœder (1902a, 49; 1903b, 631) adds no new observations.

In view of the position of the testes, as figured by Cobbold, the question naturally arises as to whether we are dealing with the same or with a different species. As our material represents some of Cobbold's material, and as various authors have been misled in interpreting the relative position of the testes, especially in the case of rather thick trematodes, we hesitate to draw the conclusion that our material represents an undescribed species. Should more of Cobbold's original material be found, or should the Indian elephant prove to harbor another amphistome which agrees with Cobbold's illustration, it will then become necessary to accept the name *papillatum* for that form and to recognize our material as representing a new species.

FIG. 191.

EXTERNAL CHARACTERS.

SIZE.—The specimens, measured in alcohol, varied in length between 4.5 and 5.5 mm. and in greatest width between 2.5 and 2.75 mm. They were, however, more or less shrunken and had evidently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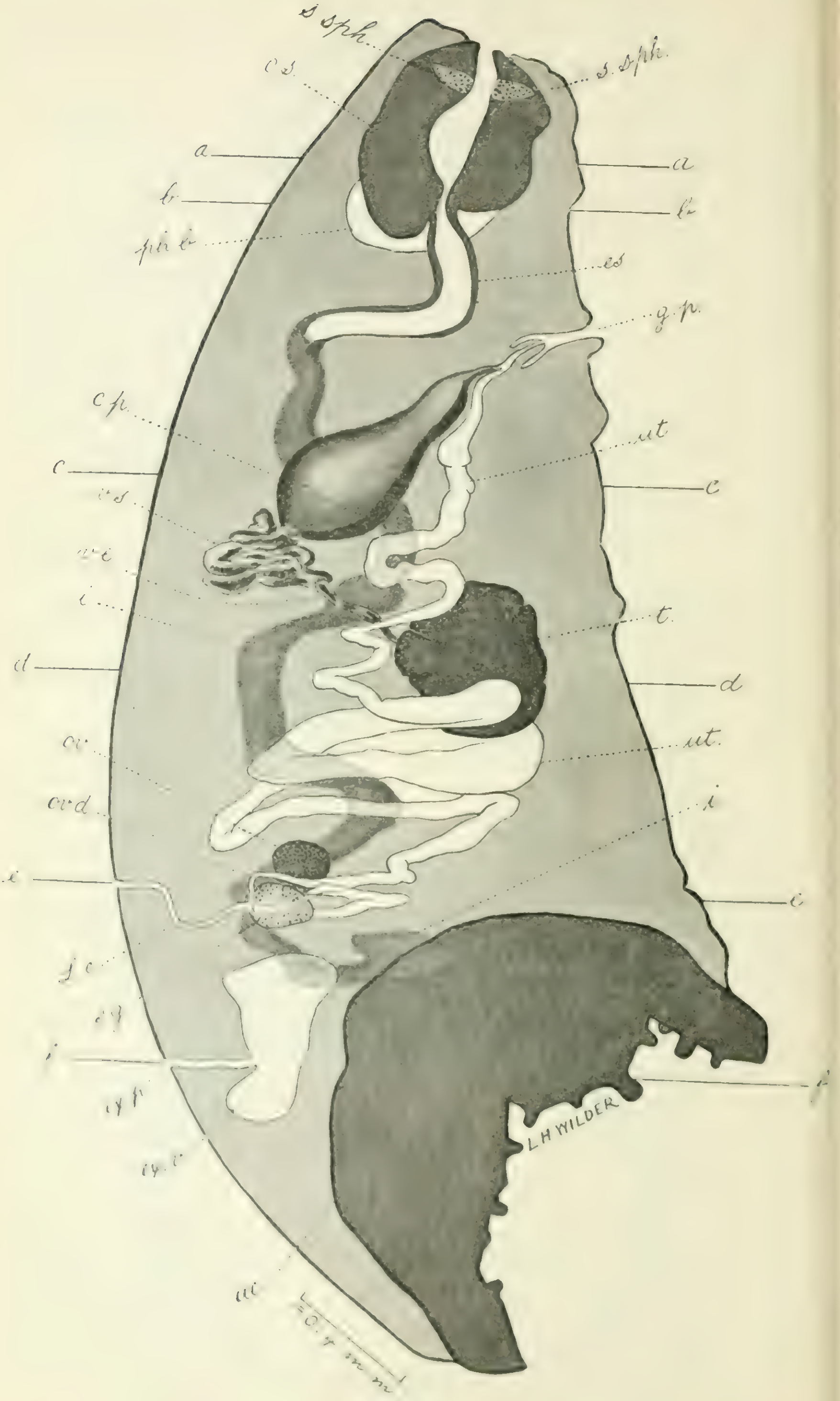
L.H.WILDER

FIG. 192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ov.d
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e
e
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i
f
ex.p
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ai
= 0.4 mm
L.H. WILDER

FIG. 193.

undergone some distortion, so that these measurements must be regarded only as approximations to the original.

After sectioning, one of the specimens measured 4.14 mm. in length, 2 mm. in greatest transverse, and 1.70 mm. in greatest dorso-ventral diameter.

COLOR.—Five of the specimens were of a pearl tint. These were slightly translucent, enabling the observer to determine the position of the testes. The remaining two specimens were of an opaque olive-green color.

FORM.—As has been stated, the specimens were obviously shrunken and variously distorted.

One of them, however, had undergone these changes to so slight a degree as to serve fairly well for the purpose of this description.

This worm (shown in figs. 190, 191) appeared somewhat conical in form, but bent slightly ventrad. Its greatest width was at the junction of the third with the terminal fourth of the body length.

From this region the body tapers in both directions; gradually and considerably toward the bluntly pointed oral extremity, slightly in the direction of the caudal extremity. The dorsum is arched both longitudinally and transversely; the venter is slightly convex from side to side, but straight or slightly concave in a longitudinal direction. The lateral margins are curved slightly both longitudinally and in a transverse plane. In transverse section the outline of the body is transversely elliptical to circular.

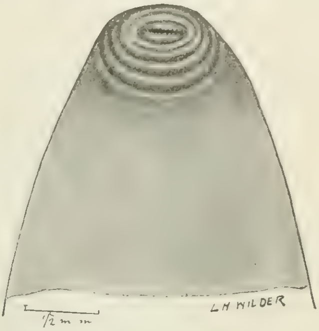

FIG. 194.

SURFACE.—The surface cuticle is without spines or scales. It is marked, however, by fine transverse striations. The bluntly pointed, attenuated extremity presents the oral aperture, which is encircled by a narrow ridge marked off from the surface by a narrow, fairly deep groove (fig. 194). Concentric with this and about equally spaced there are observed four shallow grooves (fig. 194).

Genital pore.—In the median longitudinal line of the venter, about one-fourth of the body length from the oral margin, there appears a slight, globular bulging of relatively small diameter, which presents the aperture of the genital pore. This pore leads into an elongate irregularly cylindrical chamber about 0.28 mm. long, from the fundus of which there arises a papilla, about 105μ in height and about 120μ in diameter at the base, which almost fills the corresponding portion of the lumen of this chamber. At the vertex of this (genital) papilla is the porus hermaphroditicus.

At the caudal extremity, encircling the aperture of the acetabulum and at a distance of about 0.20 mm. therefrom, there is a shallow circular groove. This groove marks the region at which the acetabulum emerges from the embrace of the caudal portion of the body of the worm. The portion of the caudal extremity of the worm between this groove and the margin of the acetabular aperture, and having somewhat the appearance of a collar, is formed by the projecting portion of the acetabulum.

Acetabulum. The acetabulum occupies the caudal extremity of the body, and, as already described, a portion emerges at the surface at the caudal extremity of the worm and forms a well-defined rim around the crateriform cavity, somewhat as in *Ps. stanleyii*, but without the deep narrow groove of the latter to mark it off. Measured from a reconstruction of a sectioned specimen the acetabulum was about 1.70 mm. in greatest transverse and 1.40 mm. in greatest dorso-ventral diameter, with an aperture 1.28 mm. in greatest transverse and 1.20 mm. in greatest dorso-ventral diameter. The cavity of the acetabulum is shallow, and this surface is beset by teat-like papillæ, some of which, in sections, measured 90*μ* in height and 60*μ* in diameter at the base.

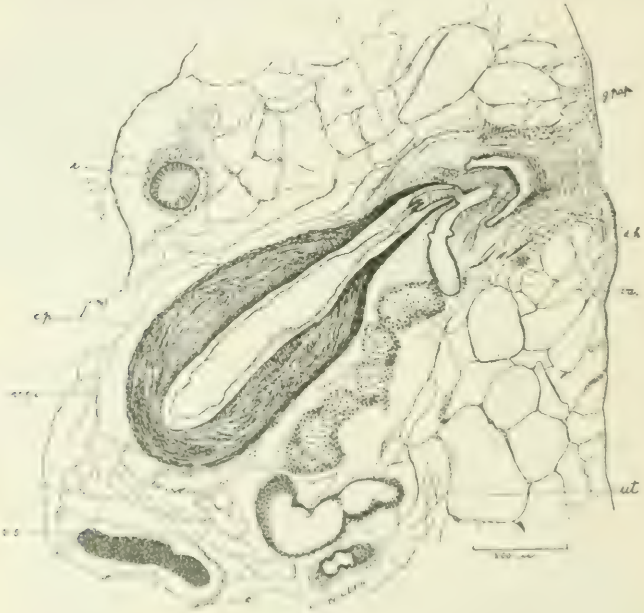

FIG. 195.

INTERNAL ANATOMY.

Digestive tract.—The bluntly-pointed cephalic extremity of the worm is pierced by the oral aperture. This aperture is transversely elliptical, and its margins are beset by small conical papillæ (fig. 194). This aperture leads directly into the oral sucker, a relatively large muscular apparatus (figs. 192, 193, 196, 197). The caudo-lateral aspects of the sucker are prolonged somewhat caudad of the transverse plane of its base into the forms of lateral bulbs. Measured from a reconstruction of one sectioned specimen, its greatest longitudinal axis was 0.64 mm., the greatest dorso-ventral diameter about 0.52 mm., and the greatest transverse diameter 0.78 mm. Measurements from sagittal sections of another specimen gave 0.66 mm. for the greatest longitudinal axis, 0.70 mm. for the greatest dorso-ventral, and about 0.72 mm. for the greatest transverse diameter. The bulbs measured from the reconstruction were about 0.40 mm. in greatest dorso-ventral and 0.30 to 0.34 mm. in greatest transverse diameter and extended about 60μ caudad of the base of the sucker.

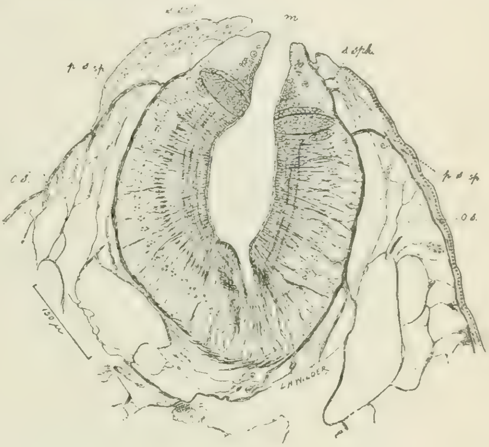

Fig. 196.

The body of the sucker appears to be inclosed in a very narrow space (fig. 196) and appears to be retained in its position by attachments at its poles and by dorso-ventral mesenterium-like and muscular bands. The long axis of the sucker coincides with the long axis of the body of the worm. In median sagittal section, the sucker has something of an oval outline with the blunt end of the oval corresponding to the base. About 120μ to 140μ caudad of the oral margin there is observed in the muscular wall a well-defined sphincter-like muscular bundle measuring about 100μ in thickness. Caudad of this sphincter the muscle walls maintain a substantially uniform thickness throughout up to the point of formation of the canal leading into the esophagus. Cephalad of the sphincter, however, the thickness of the walls becomes progressively and rapidly less, so that the muscular rim forming the oral aperture as it projects on the surface is relatively thin and sharp (fig. 196).

In transverse section the outline of the sucker is that of a blunt ellipse with its major axis in the transverse diameter of this region of the worm. Studied in median sagittal section, the base of the sucker appears slightly beveled at the expense of its ventral aspect, from which region the esophagus is seen to take its departure. On each side of and closely embracing this portion of the esophagus the caudo-lateral projections of the sucker or suctorial bulbs may be seen.

In form these bulbs are irregularly globular. Their walls, considerably thinner than those of the body of the sucker, are of relatively loose muscular mesh-like structure. The lumen of the sucker is a transversely wide, dorso-ventrally narrow space; it is directly continuous with the irregular and variable lumen of the bulbs. Both are lined by a thin cuticle-like layer.

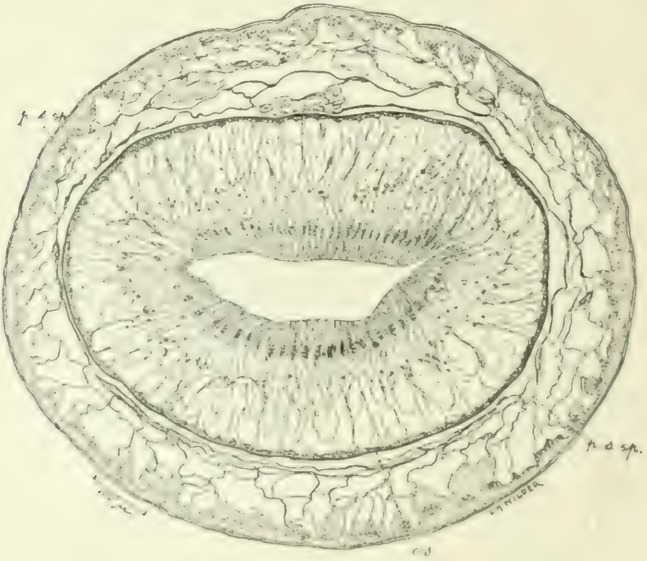

Fig. 197.

The esophagus, as already described, takes its departure from the ventral aspect of the base of the sucker. It passes at first caudad, describing a slight curve in its course with its convexity ventrad, then turns almost directly dorsad, and having reached a point about one-fourth the body length from the oral margin and about one-fourth the dorso-ventral diameter of the worm in this zone from the dorsum the esophagus curves directly caudad and almost immediately forks into two lateral intestinal tubes. This fork is slightly caudad of the genital pore. The intestinal tubes from their point of origin pass at first latero-caudad then in dorso-ventrally wavy course caudad. They finally terminate by bending abruptly ventro-cephalad in a hook-like form at a point in a transverse plane slightly caudad of that of the cephalic margin of the acetabulum.

As in the other species of this group, the esophagus is inclosed in a well-marked layer of cells. The lumen of the esophagus is lined by a cuticle-like layer, which ceases abruptly at the fork. The intestines are lined by an epithelial cell layer.

GENITAL SYSTEM.—With the exception of the vitellogene glands and the testes the genital organs are situated in the intercecal area.

Male organs.—The testes are situated in the equatorial zone on each side of the median sagittal plane and ventrad of the corresponding intestine. They are irregularly globular in form, measuring about 0.40 mm. in diameter, but with their vertical diameter a little longer than either the transverse or dorso-ventral. From their dorsal aspects, slightly above their equator, there emerges from each a vas efferens. These pass in a curved course dorso-cephalad and toward the median line to unite at about the level of the cephalic margins of the testes to form the vas deferens. The vas deferens consists first of a thin-walled, dilated, complexly coiled vesicle. This terminates abruptly in a short narrow duct, which pierces the very thick walled cirrus pouch and is continued within the latter and almost fills its lumen as a thin-walled duct (vesicula seminalis interna) to the cirrus; in a series of sagittal sections the cirrus is distinctly

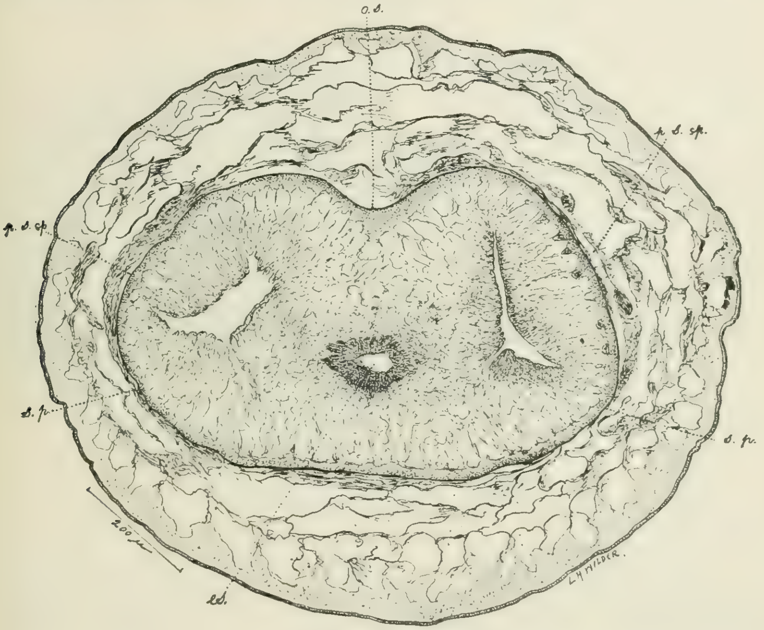

FIG. 198.

seen in an invaginated condition, but with its terminal (distal) portion pointed outward (not invaginated); the canal formed by the invaginated portion unites distally with the metraterm to form a genital cloaca apparently representing a ductus hermaphroditicus, which opens on the apex of the genital papilla. The cirrus pouch is directed obliquely ventro-cephalad; in form it suggests that of an Indian club. Its walls become gradually less thick, this reduction taking place mainly at the expense of the (internal or) longitudinal layer, and its diameter becomes progressively reduced as it passes ventro-cephalad. Its greatest diameter, measured in sections, varied in two specimens from 0.32 mm. to 0.34 mm., with a thickness of wall of 0.12 mm. In length it measured about 0.44 mm. The ductus hermaphroditicus, formed as already described, pierces the axial region of the genital papilla, on the vertex of which it opens as the porus hermaphroditicus. The presence of a distinct pars prostatica can not be definitely asserted, but in two places (within at the distal end of the cirrus pouch, and without at the proximal end) are groups of nuclei which may come into consideration in this connection if well-preserved material can be obtained.

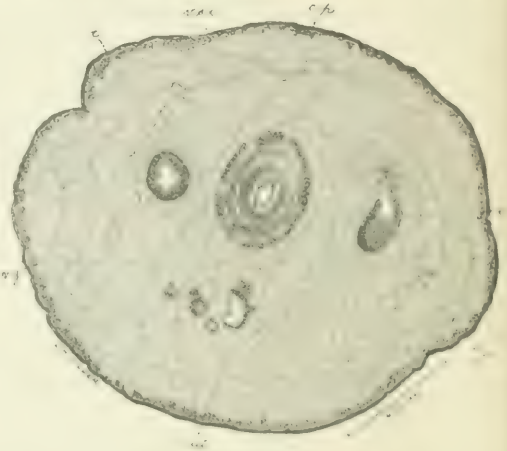

FIG. 199.

The wall of the chamber surrounding the genital papilla is provided with a well-developed muscle-complex, suggestive of the genital sucker figured by Fischœder for several species of *Cladorchis*, but the muscles are not arranged in so distinctly acetabular-like manner as Fischœder figures for the species he discusses; it therefore can not be stated that *Pfenderius papillatus* possesses a true genital sucker.

Female organs.—The ovary lies in about the median line of the body somewhat nearer the dorsum than the venter, posttesticular in a plane a little above that of the cephalic margin of the acetabulum—that is, about at the junction of the middle with the caudal third of the body. The oviduct emerges from the dorsal aspect of the ovary, passes at first directly dorsad, then curves caudad toward the dorsal aspect of the shell gland (fig. 201). The shell gland lies close to the right caudo-lateral aspect of the ovary and just at about the level of the cephalic margin of the acetabulum. On its dorso-caudal aspect it is penetrated by the oviduct and on its caudal aspect by the vitello-duct. These ducts unite to form the ootype, the continuation of which pierces the surface of the gland and emerges from its ventro-cephalic aspect as the uterus. The uterus, immediately after its emergence from the shell gland, describes numerous compact coils in front of and to the right of the shell gland, and then, as it proceeds cephalad, to the front (ventrad of) and to the right of the ovary. Beyond this point it forms some dorso-ventral coils in the space between the ceca. These coils are dilated and filled with eggs. The uterus then continues in the axial region of the body in a more direct course cephalad. Part of its course is through the space between the caudal portions of the testes, tilting later nearer the dorsum of the body to enter the interspace between the intestines, then it passes beneath the arch of union of the vasa efferentia to gain the ventral aspect of the cirrus pouch. In the remainder of its course it maintains this relation to the male duct forming but few coils, and terminates by uniting with the male duct to form the ductus hermaphroditicus.

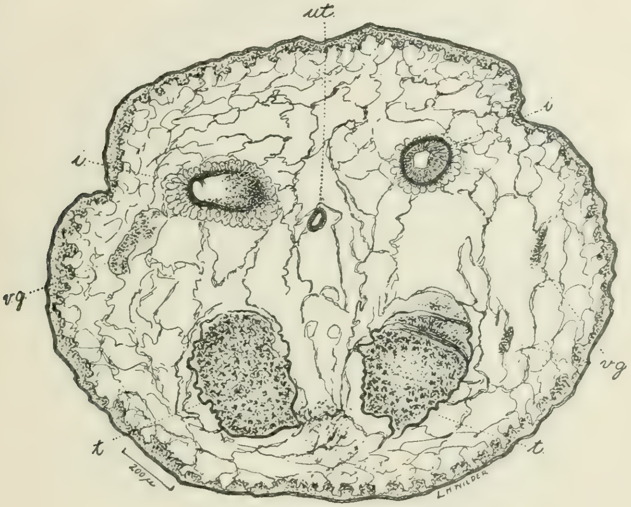

Fig. 200.

Laurer's canal takes its departure from the oviduct at a point close to the dorsal aspect of the shell gland. It then passes dorsad describing a curve in its course with its convexity caudad and opens on the dorsum somewhat to the right of the median line about 0.54 mm. cephalad of the excretory pore in a transverse plane slightly above that of the cephalic margin of the acetabulum.

The vitellogene glands consist of sparsely scattered insignificant follicles, irregularly grouped to the external, ventral, and to some extent dorsal aspect of each of the intestinal ceca. Longitudinally they extend from about the level of the esophageal fork to the level of the cecal ends of the intestines. From each of the glands a duct passes more or less transversely inward ventrally of the intestines to unite near the ventro-caudal aspect of the shell gland. From their point of union a duct passes dorsad, skirting the caudal aspect of the shell gland which it penetrates and in the substance of which it unites with the oviduct as already described.

Eggs.—Eggs were observed crowded together in some of the proximal coils of the uterus. They appeared to be elliptical in form, and 2 which appeared to have been sectioned in a favorable plane, measured each 150μ by 75μ in diameter. One end is operculated, and the opposite pole bears a short mammillate knob.

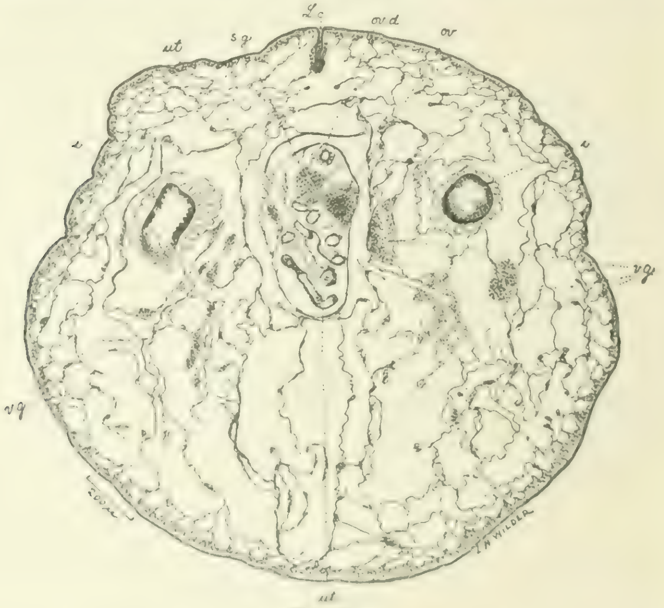

FIG. 201.

EXCRETORY SYSTEM.—The excretory system is well developed. An excretory vesicle of moderate size is situated dorsad of the dome of the acetabulum. From its dorsal aspect a little caudad of its equator a duct originates and passing directly dorsad opens in about the median line of the dorsum slightly caudad of the plane of the cephalic margin of the acetabular aperture and about 0.54 mm. caudad of the aperture of Laurer's canal. This excretory duct is about 0.24 mm. in length.

Fig. 190.—Ventral view. Enlarged. Original.

Fig. 191.—Profile view of same. Enlarged. Original.

Fig. 192.—Ventral projection to show internal anatomy. *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f–f*, planes of section; *ac.*, acetabulum; *c. p.*, cirrus pouch; *es.*, esophagus; *i.*, intestine; *ov.*, ovary; *o. s.*, oral sucker; *s. p.*, suctorial pouch; *s. sph.*, suctorial sphincter; *s. g.*, shell gland; *t.*, testes; *ut.*, uterus; *v. e.*, vasa efferentia. Slightly diagrammatic. Enlarged. Original.

Fig. 193.—Profile projection of same. *a–a*, *b–b*, *c–c*, *d–d*, *e–e*, *f f*, planes of section. *ac.*, acetabulum; *g. p.*, genital pore; *c. p.*, cirrus pouch; *es.*, esophagus; *ex. p.*, excretory pore; *ex. v.*, excretory vesicle; *i.*, intestine; *L. c.*, Laurer's canal; *ov.*, ovary; *ov. d.*, oviduct; *o. s.*, oral sucker; *s. p.*, suctorial pouch; *s. sph.*, suctorial sphincter; *s. g.*, shell gland; *t.*, testes; *ut.*, uterus; *v. e.*, vas efferens; *v. s.*, vesicula seminalis. Slightly diagrammatic. Enlarged. Original.

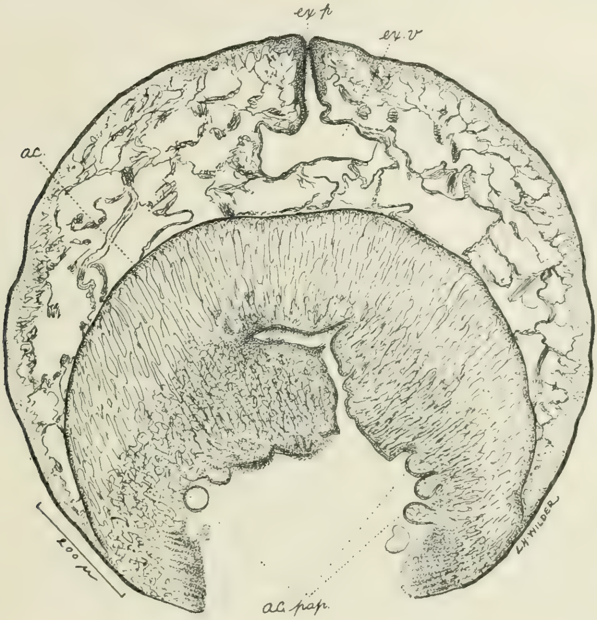

Fig. 202.

Fig. 194.—Oral extremity seen from above and in front to show concentric grooves (slightly exaggerated) around oral aperture. Compare fig. 196. Enlarged. Original.

Fig. 195.—Sagittal section through cirrus pouch (*c. p.*). Shows also the vesicula seminalis interna (*v. s. i.*), the small cirrus (*c.*), the ductus hermaphroditicus (*d. h.*), the genital papilla (*g. pap.*), the

metraterm (*ra.*), the uterus (*ut.*), a loop of the vesicula seminalis externa (*v. s.*), some loops of the uterus (*ut.*), and a section of the intestine (*i.*). Slightly diagrammatic. Enlarged. Original.

FIG. 196.—Sagittal section through oral extremity. Shows the oral sucker (*o. s.*), the suctorial sphincter (*s. sph.*), the mouth (*m.*), and the perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 197.—Transverse section at *a a* figs. 192 and 193. Shows oral sucker (*o. s.*) and perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 198.—Transverse section at *b b* figs. 192 and 193. Shows oral sucker (*o. s.*), the suctorial pouches (*s. p.*), entrance to the esophagus (*es.*), and the perisuctorial space (*p. s. sp.*). Enlarged. Original.

FIG. 199.—Transverse section at *c c* figs. 192 and 193. Shows the thick muscular cirrus pouch (*c. p.*), vesicula seminalis interna (*v. s. i.*), the intestines (*i.*), uterus (*ut.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 200.—Transverse section at *d d* figs. 192 and 193. Shows the testes (*t.*) and their relation to the intestinal ceca (*i.*) at this level, the uterus (*ut.*), and vitellaria (*v. g.*). Enlarged. Original.

FIG. 201.—Transverse section at *e e* figs. 192 and 193. Shows caudal portion of ovary (*ov.*), cephalic portion of shell gland (*s. g.*), the oviduct (*ov. d.*), Laurer's canal (*L. c.*), some uterine coils (*ut.*), intestinal ceca (*i.*), vitellaria (*v. g.*). Enlarged. Original.

FIG. 202.—Transverse section at *f f* figs. 192 and 193. Shows excretory vesicle (*ex. v.*) and pore (*ex. p.*) and acetabulum (*ac.*), with some of its papillæ (*ac. pap.*). Enlarged. Original.

Genus MICRORCHIS Daday, 1907.

GENERIC DIAGNOSIS.[a]—*Cladorchiinæ* (p. 169): Body elongate, venter concave, dorsum convex, gradually attenuate from acetabulum to mouth, caudal end rounded (on ventral view), sides rounded. Ventral pouch absent. Acetabulum large, terminal, aperture medium, directed ventrad. *Genital pore without sucker.* Excretory pore prevesicular, *equatorial*, very close to and at left of pore of Laurer's canal. Oral sucker with well-developed sphincter and with pair of evaginations; *esophagus springs centrally* from oral sucker, cephalad of evaginations, and has a *distal* muscular thickening, ceca straight, long, end far postovarial, but preacetabular.

Male organs: Testes 2, very much smaller than acetabulum, unbranched, somewhat irregular in outline, fields apparently overlap, zones separate, preovarial, preequatorial, far removed from acetabulum, interceeal; *cirrus pouch present.*

Female organs: Ovary and shell gland *preequatorial*, at equator of vitellaria, posttesticular, vitellaria near ceca, equatorial, nearly one-third as long as body, "branched, tree-like;" Laurer's canal prevesicular.

TYPE.—*M. megacotyle* (Diesing, 1836).

Genus CHIORCHIS Fischœder, 1901.

GENERIC DIAGNOSIS.[b]—*Cladorchiinæ* (p. 169): Body straight, venter flat, dorsum convex, cephalic end attenuate, caudal end rounded, sides sharp, not divided by constriction. Ventral pouch absent. Acetabulum distinctly ventral, relatively small, extends beyond surface, margin raised, aperture large. Genital pore without sucker, ductus hermaphroditicus present. Excretory pore prevesicular, preacetabular, caudad of Laurer's canal. Oral sucker with paired evaginations; esophagus with pronounced distal muscular thickening; ceca nearly straight, end postequatorial, posttesticular.

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[a] Based on Daday, 1907.

[b] Based on Fischœder, 1903h.

Male organs: Testes 2, slightly smaller than acetabulum, each with 4 lobes forming cross on ventral view, fields coincide, zones separate, preovarial, in equatorial and caudal thirds; musculosa not enormous; cirrus pouch present.

Female organs: Ovary and shell gland posttesticular; vitellaria in extracecal and cecal areas, extend through part of esophageal and entire cecal zones, close to ceca; uterus intercecal; Laurer's canal, chiefly prevesicular, does not cross excretory vesicle or canal.

Type.—*C. fabaceus* (Diesing, 1838).

Habitat.—Small and large intestines of marine mammals.

Genus BALANORCHIS[a] Fischœder, 1901.

Generic diagnosis.—*Cladorchiinæ* (p. 169): Body small, rather conical, venter slightly concave, dorsum convex, cephalic end attenuate, caudal end slightly attenuate but rounded, transverse section circular. Ventral pouch absent. Acetabulum small, terminal, not sunken, margin (?), aperture relatively large. Genital pore with muscular ring; no ductus hermaphroditicus. Excretory pore postvesicular, posttesticular, apparently near cephalic margin of acetabular zone, caudad of pore of Laurer's canal. Oral sucker with paired evaginations; esophagus without muscular thickening; ceca long, slightly wavy, end postequatorial, in or near acetabular zone. Genital papilla absent.

Male organs: Testes 2, larger than acetabulum, elongate-elliptical, fields separate, zones nearly coincide, chiefly postovarial, postequatorial, close to acetabulum, cirrus pouch present.

Female organs: Ovary and shell gland in cephalic half of testicular zone; vitellaria S-shaped, following ceca, extend through nearly entire cecal zone, follicles united in globular groups; uterus slightly developed, intercecal, almost entirely pretesticular, not posttesticular; metraterm opens on caudal margin of pore, caudad of cirrus pouch.

Type.—*B. anastrophus* Fischœder, 1901.

Habitat.—First stomach of *Cervidæ*, Brazil.

Subfamily DIPLODISCINÆ Cohn, 1904.

Subfamily diagnosis.[b]—*Paramphistomidæ* (p. 60): [The characters of this subfamily are still in some doubt. If the excretory system of *Diplodiscus* is characteristic for the entire group, that would make an excellent subfamily character.]

Key to Genera of Diplodiscinæ.

Acetabulum round, opens caudad, with central excavation; 2 testes in young, coalesce in adult; genital pore near mouth; esophagus long, straight, with muscular thickening ("pharynx") at bifurcation; excretory canals with dark concretions.
Diplodiscus, p. 248.

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[a] Based on Fischœder, 1903h.

[b] *Original diagnosis.*—Amphistomiden von gedrungener, konischer Form und runden Querschnitt. Mundsaugnapf gut ausgebildet, mit 2 retrodorsalen Taschen. Ein grosser Endsaugnapf, ueber welchem dorsal der Exkretionsporus liegt. Mundœffung terminal, Darmschenkel bis zum Endsaugnapf reichend, relativ sehr breit. Leben im Enddarm von Amphibien und Reptilien.—Cohn, 1904, 242.

Acetabulum elongate-oval, ventro-subterminal, divided into 2 parts by constriction; 1 testis; genital pore slightly postbifurcal; esophagus long, straight, with muscular thickening ("pharynx") at bifurcation; excretory canals with dark concretions. *Catadiscus*, p. 248.

Acetabulum round, opens caudad, with central projecting sucker; 2 testes in adult; genital pore near mouth; esophagus short, bent, without muscular thickening ("pharynx"); excretory canals without concretions........*Opisthodiscus*, p. 248.

Genus DIPLODISCUS Diesing, 1836.

GENERIC DIAGNOSIS.[a] [b]—*Diplodiscinæ* (?) *or Cladorchiinæ* (?) (p. 247): Body conical to cylindrical, venter concave, dorsum convex, cephalic end attenuate (bluntly pointed), caudal end obliquely truncate. Ventral pouch absent. Acetabulum terminal, very large, aperture large, tilted ventrad. Genital pore without sucker. Excretory pore postvesicular, in acetabular zone, caudad of Laurer's canal; radial excretory branches profuse in acetabulum. Oral sucker with paired evaginations; esophagus with distal muscular thickening; ceca straight, long, end postequatorial, posttesticular.

Male organs: Testes 2 in young; may unite in adult, interceecal, much smaller than acetabulum, globular, unbranched, preovarial, rather distant from acetabulum, about equatorial; cirrus pouch present.

Female organs: Ovary and shell gland largely posttesticular; vitellaria extend from esophageal into postcecal zone; uterus interceecal; eggs with operculum; Laurer's canal entirely prevesicular.

TYPE.—*D. subclavatus* (Gœze, 1782).

HABITAT.—In rectum of amphibians, Europe.

Genus OPISTHODISCUS Cohn, 1904.

GENERIC DIAGNOSIS.[c]—*Diplodiscinæ* (?) (p. 247): Acetabulum with central projecting sucker. Esophagus without pharyngeal swelling.

Male organs: Testes 2, in part postovarial; cirrus pouch long and narrow.

Female organs: Ovary in testicular zone.

TYPE SPECIES.—*O. diplodiscoides* (Cohn, 1904).

Genus CATADISCUS Cohn, 1904.

GENERIC DIAGNOSIS.[d]—*Diplodiscinæ* (?) (p. 247): Acetabulum divided into 2 halves. Esophagus with distal muscular thickening.

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[a] *Diplodiscus* after Cohn, 1904, 242. *Diplodiscinæ:* Der runde Endsaugnapf ist nach hinten gerichtet, mit zentraler Exkavation. 2 Hoden, die bei alten Exemplaren verschmelzen. Genital porus ziemlich nahe der Mundöffnung. Oesophagus lang und gerade verlaufend, ein Pharynx an der Darmgabelung. Exkretionskanaele mit den typischen dunkeln Konkrementen.

[b] Combined from various authors.

[c] *Original diagnosis. Diplodiscinæ:* Der runde Endsaugnapf ist nach hinten gerichtet, mit centralem vorragendem Zapfen. Dauernd 2 Hoden. Genitalporus nahe an der Mundöffnung. Oesophagus kurz, um den Mundsaugnapf herum gebogen. Kein Pharynx. Die mächtige entwickelten Saugnapftaschen reichen bis zu ⅓ der Gesammtlänge. Darm asymmetrisch. Exkretionskanal ohne dunkele Konkretion.—Cohn, 1904, 243.

[d] *Original diagnosis. Diplodiscinæ:* Der langovale Endsaugnapf liegt subterminal-ventral und ist durch Einschnürung in 2 Theile geteilt. Ein Hoden. Genitalporus wenig vor der Körper medial, dicht hinter der Darmgabelung. Oesophagus lang, gerade verlaufend, ein Pharynx an der Darmgabelung. Exkretionskanäle mit typischen Konkretionen. Cohn, 1904, 243.

Male organs: Cirrus pouch present. Testis single, median.

Female organs: Ovary, at least in part posttesticular.

TYPE SPECIES.—*C. dolichocotyle* (Cohn, 1903).

GASTRODISCIDÆ, new family.

FAMILY DIAGNOSIS.—*Paramphistomoidea* (p. 15): Body rather discoidal, divided by transverse constriction into cephalic and caudal portions; ventral pouch absent, venter with many large papillæ. Acetabulum ventral at caudal end.

TYPE GENUS.—*Gastrodiscus* Leuckart, 1877.

This family contains two genera (*Gastrodiscus* and *Homalogaster*), which differ so strikingly from the genera of the *Paramphistomidæ* that distinct family rank seems justified. The one point which speaks against eliminating them from *Cladorchiinæ*, *Paramphistomidæ*, is the paired evaginations of the oral sucker.

The genera may easily be separated by the following key:

Genital glands confined to large anterior portion; acetabulum moderate; type *paloniæ*..*Homalogaster*, p. 249.

Genital glands confined to large caudal portion; acetabulum small; type *sonsinoii*=*ægyptiacus*..*Gastrodiscus*, p. 252.

Genus HOMALOGASTER Poirier, 1883.

GENERIC DIAGNOSIS.—*Gastrodiscidæ* (p. 249): Body divided by constriction into large cephalic, flat portion with genital glands, and small, caudal portion with acetabulum; venter flat or excavate, provided with alternating longitudinal rows of large mamma-like structures; of these, the papillæ near the middle of the rows (both longitudinal and transverse) are larger than those further from the middle; dorsum convex. Acetabulum large, caudal, ventral, not sunken, margin raised, aperture medium. Genital pore without sucker, on large protrusile papilla, ductus hermaphroditicus absent. Excretory pore postvesicular in acetabular zone, caudad of pore of Laurer's canal. Oral sucker with paired evaginations; esophagus rather long, with muscular swelling (at least in *H. philippinensis*); ceca slightly wavy, long, end postequatorial, posttesticular.

Male organs: Testes 2, smaller than acetabulum, preequatorial, lobate, widely separate from ovary and acetabulum; musculosa not enormously developed; cirrus pouch absent.

Female organs: Ovary and shell gland posttesticular; vitellaria cecal, about from bifurcation to ovary; uterus intercecal, pre-, post-, and testicular; eggs operculated; Laurer's canal entirely prevesicular.

TYPE.—*H. paloniæ* Poirier, 1883.

HABITAT.—Large intestine of ruminants. Asiatic.

HOMALOGASTER PHILIPPINENSIS Stiles & Goldberger, 1908.

[Figs. 203–205.]

A reexamination of the sectioned material that was used as the basis for the description of this species by Stiles and Goldberger, 1908, has disclosed one or two additional interesting points.

Oral sucker.—The oral sucker and suctorial pouches are inclosed in a well-marked cavity (figs. 32–36, Stiles and Goldberger, 1908). The oral sucker is held in position by attachments around its oral extremity and by mesenterium-like strands extending from its ventral and dorsal aspects to the parenchyma. Caudally of the base of the oral sucker similar dorso-ventral strands run from the dorsomesial aspects of the bulbs toward the dorsum, anchoring them to the parenchyma, and other strands run ventrad from the esophagus. Besides these mesenterium-like strands, consisting of a loose parenchyma-like membrane, there are muscle bundles which attach these structures to the subcuticular muscle layers ventrally and dorsally. In this cavity or perisuctorial space is a considerable amount of a granular material (coagulum) in which a few cell-nuclei are scattered. These nuclei are surrounded by a narrow, clear, nongranular area, but a well-defined cell wall could not be made out.

Esophagus.—The caudal extremity of the esophagus is characterized by a somewhat fusiform bulbous enlargement (fig. 203) due to a very marked thickening of the muscular layers. Measured in sagittal section this bulbous thickening is about 280μ long with a maximum thickness of wall of about 67μ. The thickness of the esophageal wall just above (cephalad of) the beginning of this bulbous enlargement was found to be, in the same section, about 15μ.

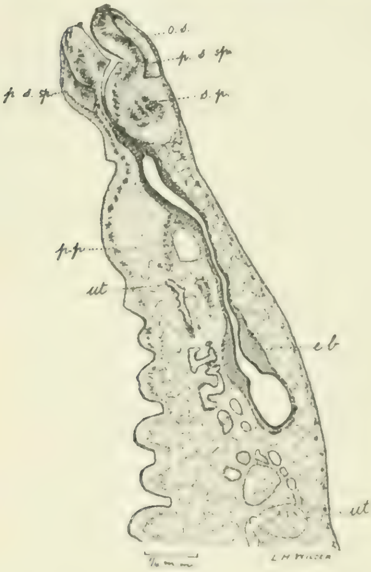

FIG. 203.

Acetabulum.—The rim of the acetabulum projects beyond the body parenchyma, which embraces its dome in a manner resembling somewhat that in *Pseudodiscus stanleyii,* though it is not marked off as in *stanleyii* by an encircling groove (figs. 204, 205).

The foregoing observations change the original specific diagnosis to read as follows:

SPECIFIC DIAGNOSIS.—*Homalogaster* (p. 249): Body 7.5 to 9 mm. long by 4.5 to 5 mm. (or flattened to 7 mm.) broad, canoe shaped, cephalic extremity attenuated, caudal extremity rounded; when flattened, sides very convex longitudinally; dorsum may show submedian longitudinal depressions. Genital pore 1 mm. from oral margin (about one-ninth to one-seventh of body length from mouth and about at equator of esophagus), surrounded by depressed circular area bearing numerous small papillæ. Venter with alternating longitudinal rows of large papillæ extending from about 2 to 2.5 mm. from oral margin to acetabulum. Margins curved ventrally and are fairly sharp. Acetabulum about 2.5 by 2.7 to 3 mm., its margin projects beyond the body parenchyma. Oral sucker with a single pair of evaginations; the sucker and evaginations lie in a well-marked perisuctorial space; esophagus extends to about one-fifth of body length from oral margin; the caudal end of the esophagus is provided with a fusiform bulbous muscular enlargement about 280μ long by 67μ thick; intestinal ceca narrow, slightly tortuous, long, extending to acetabulum. Excretory pore postvesicular, dorso-median, about at equator of acetabulum, caudad of pore of Laurer's canal.

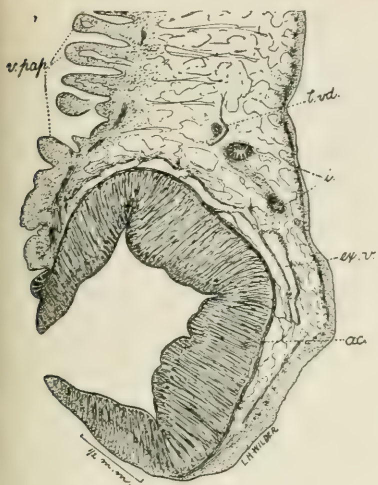

FIG. 204.

Male organs: Testes lobate in cephalic half of space between ceca; one caudad of the other; vesicula seminalis quite compactly coiled; pars musculosa distinctly but not highly developed; pars prostatica not prominent, may enlarge to quite a large diameter, thus resembling a "vesicula seminalis interna;" ductus ejaculatorius present; cirrus absent.

Female organs: Ovary and shell gland submedian, near end of one of the ceca, ovary cephalad of shell gland; vitellaria extend about from the bifurcation of the esophagus to caudal plane of ovary; uterus with many coils, well developed, passes cephalad, dorsally of testes, ventrally between vasa efferentia, ventrally of vas deferens, to pore; Laurer's canal opens dorso-median at plane of termination of ceca. Genital papilla, when extruded, resembles human penis with glans; bears on its vertex a pore, from which runs the short ductus hermaphroditicus; when retracted the papilla resembles a partially inclosed cirrus pouch.

Eggs: Egg oval, operculated, with small knob at opposite pole; 125 to 139μ by 67.5 to 86μ.

HABITAT.—Cecum of *Bos* sp., at Manila, P. I. (type locality), and Phrapatoom, Siam.

TYPE.—U.S.P.H. & M.H.S. 9580 (mounted); Cotypes 9581 and 9960.

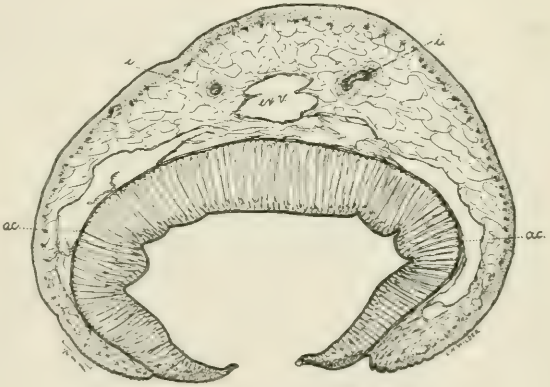

FIG. 205.

ILLUSTRATIONS.

FIG. 203.—Sagittal section shows bulbous thickening of caudal end of esophagus (*e.b.*); oral sucker (*o.s.*); perisuctorial space (*p.s.sp.*); suctorial pouch (*s. p.*); pars prostatica (*p. p.*); uterus (*ut.*). Enlarged. Original.

FIG. 204.—Sagittal section of caudal extremity to show acetabulum (*ac.*). Shows also the excretory vesicle (*ex. v.*) and intestines (*i.*), section of transverse vitello-duct (*t. vd.*), and ventral papillæ (*v. pap.*). Enlarged. Original.

FIG. 205.—Transverse section through caudal extremity. Shows acetabulum (*ac.*), excretory vesicle (*ex. v.*), and intestines (*i.*). Enlarged. Original.

Genus GASTRODICUS Leuckart, 1877.

GENERIC DIAGNOSIS.[a]—*Gastrodiscida* (p. 249): Body divided by constriction into small, cephalic, slender, nearly cylindrical portion without sexual glands, and large, caudal, flat, discoidal, ventrally excavate portion containing the genital glands. Ventral pouch absent. Acetabulum small, caudal, ventral, margin raised, aperture relatively large. Genital pore without sucker. Excretory pore postvesicular, in acetabular zone, caudad of pore of Laurer's canal. Oral sucker with paired evaginations; esophagus with muscular thickening; ceca not wavy, long, end postequatorial, posttesticular.

Male organs: Testes 2, larger than acetabulum, branched, diagonal, fields and zones overlap, preovarial, widely separated from acetabulum, about in equatorial third; musculosa not enormous; "cirrus pouch not completely closed."

Female organs: Ovary and shell gland posttesticular; vitellaria extraceecal, extend from bifurcation into postcecal zone; uterus interceecal; Laurer's canal entirely prevesicular.

TYPE.—*Gastrodiscus ægyptiacus* Cobbold, 1876.

HABITAT.—Small and large intestines of *Equus* in Egypt and India, and *Homo* in India.

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[a] Based upon the writings of various authors.

LIST OF ABBREVIATIONS.

a. ac......aperture of acetabulum.
a. v. p....aperture of ventral pouch.
ac........acetabulum.
ac. pap....acetabular papillæ.
c.........cirrus.
c. p.......cirrus pouch.
c. vd......common vitello-duct.
cu........cuticle.
d. ac......dome of acetabulum.
d. e.......ductus ejaculatorius.
d. h.......ductus hermaphroditicus.
e. g.......brain (esophageal ganglion).
es.........esophagus.
es. b......esophageal bulbous thickening.
es. f.......esophageal fork.
ex. c......excretory canal.
ex. d......excretory duct.
ex. p......excretory pore.
ex. v......excretory vesicle.
g. a. c.....ventral chamber of genita atrium.
g. a.......genital atrium or dorsal chamber of genital atrium.
g. b.......genital bulging.
g. pap.....genital papillæ.
g. p.......genital pore.
g. s.......genital sucker.
g. sph.....genital sphincter.
i.........intestine.
L. c.......Laurer's canal.
m.........mouth.
m. b......mesenterium-like strands (of oral sucker).
m. p......musculosa-prostatica, junction of.
o. pap.....oral papillæ.
o. s.......oral sucker.
ot........ootype.
ov........ovary.
ov. d......oviduct.
p. i.......pars intermedia.
p. L. c....pore of Laurer's canal.
p. m......pars musculosa.
po. gr.....peri-oral groove.
por. h.....porus hermaphroditicus.
p. p.......pars prostatica.
pap. o. s. .papillæ of oral sucker.
p. s. sp....perisuctorial space.
pa. gr.....peri-acetabular groove.
pap. g. a. .papillæ of genital atrium.
par.......parenchyma.
r. ac......rim of aperture of acetabulum.
s. b.......suctorial bulb.
s. g.......shell gland.
s. p.......suctorial pouch.
s. pap.....surface papillæ.
s. sph.....suctorial sphincter.
sz.........spermatozoa.
t.........testis (t. d., right or inferior t. s., left or superior).
t. g. p.....true genital pore.
tr. gr......transverse grooves.
t. vd......transverse vitello-duct.
ut........uterus.
v. e.......vas efferens (right = v. e. d.; left=v. e. s.).
v. e. a.....arch of union of vasa efferentia.
v. d.......vas deferens.
v. g.......vitellogene glands, vitellaria.
v. m......vesicula-musculosa, junction of.
v. p.......ventral pouch.
v. pap....ventral papillæ.
v. r.......vitellene reservoir.
v. s.......vesicula seminalis.
v. s. i.....vesicula seminalis interna.
va ....metraterm.

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LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi.* By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

*No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

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*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

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No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

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*No. 41.—Milk and its relation to the public health. By various authors.

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No. 60.—A study of the anatomy of *Watsonius* (n. g.) *watsoni* of man, and of 19 allied species of mammalian trematode worms of the superfamily *Paramphistomoidea*. By Ch. Wardell Stiles and Joseph Goldberger.

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TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN NO. 61

JANUARY, 1910

QUANTITATIVE PHARMACOLOGICAL STUDIES:

RELATIVE PHYSIOLOGICAL ACTIVITY OF SOME COMMERCIAL SOLUTIONS OF EPINEPHRIN

By

W. H. SCHULTZ

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WALTER WYMAN, *Surgeon-General.*

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QUANTITATIVE PHARMACOLOGICAL STUDIES: RELATIVE PHYSIOLOGICAL ACTIVITY OF SOME COMMERCIAL SOLUTIONS OF EPINEPHRIN.[a]

By W. H. Schultz,

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A cursory reading of the clinical literature on epinephrin shows conflicting opinions held regarding the size of the dose necessary to produce a physiological effect. Indeed, it will also be observed that in many cases negative results are unexpectedly obtained. Obviously, with a drug like epinephrin, given in critical cases, it is of importance that its degree of effectiveness be ascertained.

In a previous paper it has been shown that a pure sample of epinephrin base furnishes a reliable standard in assaying vaso-constrictor substances. This epinephrin base can now be made so as to contain practically no ash. Its solution can be made up as needed so as to show but a trace of color, with a maximum physiological and optical activity and keeping power that meets all the requirements of a standard. With the data now at hand it seems that the physiological activity of all epinephrin-like bodies can be assayed by the blood-pressure method and their efficiency expressed in terms of pure epinephrin base.

Hence if one has two solutions, a known epinephrin solution, 1 c. c. of which contains a grams of base, and an unknown containing a certain amount, y, of vaso-constrictor exciting substance, they can readily be checked against each other by the blood pressure method. Whichever solution is the stronger can be diluted until 1 c. c. injections of it cause the same rise of blood pressure as the other, and finally their relative activity calculated by the following equation:

$$\frac{a \text{ epinephrin base}}{y \text{ vaso-constrictor excitant}} = \text{relative activity.}$$

HISTORICAL.

Houghton, 1901 (4) suggests the use of pharmacologic methods for assaying galenical preparations and takes advantage of an already well known fact that a graduated rise of blood pressure could be obtained by injecting increasingly large doses of epinephrin intra-

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[a] Manuscript submitted for publication November 8, 1909.

venously. In 1902 (5) he proved the blood-pressure method to be one of the most accurate methods for assaying epinephrin preparations. Three solutions, A, B, and C, were used, one containing 85, one 40, and another 130 per cent of the amount of epinephrin known to be in a given solution. An assistant reported that A contained 80, B 40, and C 135 per cent of the amount in the known solution. One of Takamine's first samples of the crystalline product was tested and found to be 600 to 800 times as active as the best aqueous extracts of freshly prepared glands.

Cameron, (1) 1906, was one of the first to apply the blood-pressure method to the standardization of commercial preparations of epinephrin. After trying the colorimetric test described by Battelli, the frog profusion method described by Läwen, and the frog's pupil method proposed by Meltzer and described more in detail by Ehrmann, he finally decided on the blood-pressure method as the most convenient and reliable pharmacological assay for epinephrin. Since no standard preparation of epinephrin base was at hand, Cameron proposed the use of a drug that produced a rapid and quantitative vaso-dilator effect. Advantage being taken of the antagonistic action known to exist between epinephrin and the nitrites, he decided to check the action of definite doses of a nitrite against the epinephrin solution to be tested. Using nitroglycerin as a standard, he found that when 0.5 c. c. of 1.5 per cent 1:1000 adrenalin solution was injected along with 1 c. c. of solution containing 0.6 milligram of nitroglycerin the epinephrin rise was almost entirely cut down, and the opposing effect of the nitroglycerin was soon manifested by a very slight fall and then a return to the normal pressure. That is, 0.0075 milligram of epinephrin is antagonized by 0.6 milligram of nitroglycerin, the relative opposing action of the nitrite and the epinephrin being to each other as 80 to 1. By this method Cameron found adrenalin, suprarenalin, and hemisine to be of uniform strength. Certain other preparations not named were also tested, but were found to be uncertain in their action, some samples even causing a fall of pressure instead of a rise.

In this country the first practical demonstration of the advisability of testing the various commercial samples of epinephrin is brought out in the results published by Hunt and by Sollmann.

Hunt, 1906 (6), tested a number of epinephrin samples submitted to one of the departments of the United States Government along with certain samples bought upon the open market. In the first series samples A, B, and C were tested. Two bottles, each labeled 1:1000, were bought upon the open market and their activity tested upon rabbits anesthetized with urethane and chloralhydrate, both vagi being cut, atropin given, and, finally, the animal curarized. Under these conditions solutions A and B showed equal activity, producing, however, from 1.4 to 1.8 times the rise of blood pressure as did an equal amount of C. And when equal amounts of solutions A and B and of C were injected it was necessary to make C 1.7 times as concentrated as A and B in order to produce like rises of blood pressure. At about the same time four preparations were submitted by competing firms to another department of the Government, which in turn sent them to the Hygienic Laboratory for testing. A, C, and D purported to be 1:1000 solutions, and B suprarenal gland (dissicated), A and C being made by the same firm. These two were found to be about equal in activity, but five times as active as D, although D was offered at about one-third the price of the former. One ounce of B made up into a solution made the equivalent of about 15 ounces of A, the cost of the former being 60 cents and the latter 72 cents.

Sollmann and Brown, 1906 (9), tested four preparations of epinephrin bought upon the open market in Cleveland, Ohio. Series "*a*" was bought from a jobbing druggist and series "*b*" from a high class prescription pharmacy. "In all, 71 injections were made on 12 dogs." In each case 1 to 50,000 solutions were injected into the femoral vein of anesthetized dogs. Sometimes the vagus was cut, at other times not. If solution 2*a* be taken as a standard equal to 100, it was found that fresh dilutions of the samples tested showed the following relative efficiency:

	Efficiency of *a*.	Efficiency of *b*.
Preparation 1	70	70
Preparation 2	100	86
Preparation 3	86	63
Preparation 4	95	Nil.

The keeping quality was estimated by allowing 1:50 dilutions of 1:1000 solutions to stand a specified time and then observing the physiological efficiency and the change of color. The efficiency of the preparations varied greatly; taking the best sample as 100, the others were found to have a value of 95, 86, 70, 63, and 0.

The stability of the various samples was more constant, but all of them when diluted were practically worthless after 24 hours' standing, as will be seen in the following table:

Preparation.	Efficiency of fresh solution.	After standing one day.	After two days.	Discoloration in— One day.	Discoloration in— Two days.
1*a*	70	25	0	None	
1*b*	70	Active.	Active.	None	None.
2*a*	100	0		Dark pink	
2*b*	86	0		Dark pink	
3*a*	86	15	0	None	
3*b*	63	0		None	None.
4*a*	95	0		Light pink	
4*b*	0			Light pink	

In view of the deterioration of such preparations as the above, a dating and returning system similar to that in vogue for antitoxin sera is suggested.

Crawford, 1907 (2), gives an excellent discussion of the various methods for testing epinephrin solutions, and calls attention to the necessity of additional work along the lines of physiological assay.

Gunn and Harrison, 1908 (3), made a rather careful study of the factors supposed to influence the rate of deterioration of epinephrin solutions. The effect of acids and traces of alkalies and iron were also tested. In addition the influence of atmospheric oxygen and of light were tested upon solutions put up in plain and paraffined containers. A part of these solutions was then tested by the blood-pressure method and the relative activity determined. Solutions in full bottles containing twice the minimal amount of HCl necessary to dissolve the base were twice as active as those containing only a minimum amount of HCl. After 14 days the former showed no color, whereas the latter were a dark red. Solutions in saturated boric acid free from iron kept 8 weeks, and were twice as active as similar solutions in ordinary medicinal boric acid. Some solutions 7 months old were deep red in color and were inactive. Solutions kept in bottles only half full invariably changed color.

EXPERIMENTAL.

In testing the relative physiological activity of epinephrin and epinephrin-like compounds it has been found that dogs yield the most constant and uniform results. The method used is practically that described in Bulletin 55 of the Hygienic Laboratory.

Heretofore in purely scientific investigations there seems to have been no attempt to use a standard preparation of epinephrin base as a unit in determining the relative activity of commercial vaso-constrictors. Yet the relative rise of blood pressure after alternate and successive intravenous injections of a 1:50,000 solution of pure epinephrin base and an equivalent amount of the drug to be standardized is the most accurate method of physiological assay at present known. At least, all epinephrin-like bodies can be thus assayed, and other vaso-constrictor stimulants might also be compared with it and referred to in terms of epinephrin base. The optical activity of the standard epinephrin base ought to be known and its purity assured. When once a pure sample is obtained it ought to be kept in a dark, dry place in a container well sealed. How long such a sample retains its original activity remains to be determined. The sample used here has shown no deterioration, though it has been kept over a year. All standard solutions should be made up as needed, preferably 1 gram of base to 1,000 c. c. of normal saline. The saline solution being acidulated with twice the calculated amount of HCl necessary to form the chloride. This stock solution is then diluted as needed, 10 to 20 c. c. of the required dilution being made at a time. In the present experiment this standard 1:1,000 solution was labeled A and kept under exactly the same conditions as the commercial preparations to be tested. In order to eliminate the possibility of bias, all preparations were marked by letters, so that the person testing them did not know the brand tested until after the results had been tabulated. It was only after all of the tests had been made and the tables herein contained were made out that the names became known and were prefixed to the letters as indicated below.

The commercial preparations are in two series. One series, "*d*," received directly from the manufacturers and marked B^d, C^d, E^d, etc. The other series, "*m*," composed of the same preparations, was bought on the open market and labeled B^m, C^m, E^m, etc. Each of series "*d*" was first tested in such a manner that 1 c. c. injections of a given sample yielded the same rise of blood pressure as 1 c. c. of A. If, perchance, 1 c. c. of a 1:100,000 solution of A caused the same rise of blood pressure as did 1 c. c. of a 1:100,000 solution of B^d, then a concentration of A and B^d were checked against each other. If the individual readings of each set agreed, nothing more was done, as a rule, than to duplicate readings. On the other hand, if the solution B^d proved weaker than A, then A was diluted or a stronger solution of B^d was made, and so on until a concentration was found at which 1 c. c. injections of each of the two solutions caused the same rise of blood pressure. Then the concentration of each was doubled or halved and a second set of readings taken as a check.

In the first series of tests labeled A^1, B^{d1}, B^{m1}, etc., the commercial samples were not opened until the morning of the experiment, at which time three to four samples were opened and 2 c. c. measured out into a clean ground-glass stoppered bottle by means of a steril 2 c. c. pipette graduated in 0.05 c. c., three samples of each being taken. Samples A^1, B^{d1}, C^{d1}, and E^{d1} were then diluted as needed, to 1:10,000, and further dilutions made from this in the course of the day's experiment, 5 to 10 c. c. of each sample being made just before using for a given set of injections. The other numbers of the set, A^2, B^{d2}, A^{a3}, B^{d3}, etc., were put away in a dark, cool place for later observations. If the solution when diluted showed no color, great care was taken not to compare a diluted sample that later showed a trace of color with a sample not showing any color. On the other hand, if the original 1:1,000 solution was colored, and consequently the 1:10,000 dilution colored, I endeavored to work with dilutions corresponding to the diluted tint of the 1:10,000, but compared it with colorless A. In other words, some solutions showed a greater tendency to turn red and even brownish more quickly than others, but the quantities of such a solution made up at a given time were such as to give it every advantage possible in reading its greatest strength.

RESULTS OBTAINED BY COMPARING COMMERCIAL EPINEPHRIN SOLUTIONS WITH A STANDARD SOLUTION OF EPINEPHRIN BASE.

A. SERIES *D* RECEIVED FROM THE MANUFACTURERS OR THEIR AGENTS.

In Table I A it will be seen that 1 c. c. of a 1:200,000 solution of epinephrin base causes the same rise of blood pressure as does 1 c. c. of a 1:100,000 solution of adnephrin. And in part B of the same table a 1:100,000 solution of epinephrin base is the equivalent of a 1:50,000 adnephrin solution. From these results it would seem that the adnephrin solution contains only one-half the active principle indicated on the label. Judging from the comparative results given in Table XIV the keeping property of this solution is good, since the changes in color and in physiological activity undergone in the course of 10 days, after having been diluted to one-tenth its labeled strength, is but slight.

TABLE I. *Showing rises of blood pressure following intravenous injections of adnephrin and epinephrin.*

A.

Blood-pressure experiment No. 1, September 8, 1909. Male dog, weight 8.1 kilograms, subcutaneous injection of 81 milligrams morphine sulphate, ether anesthesia. Both vagi cut. Artificial respiration of warm air. Small doses of curare. Solutions injected into right and left saphenous veins. Epinephrin base used as a standard, being made up fresh on the morning of the experiment.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	49	½	....	....	4.13	....	....	....
Adnephrin solution B^{d}	66	1	1	100,000	4.48	137.0	170.4	33.4
Epinephrin solution A^{a}	67	1	1	200,000	4.49	136.4	170.2	33.8
Adnephrin solution B^{d}	78	1	1	100,000	5.17	153.6	187.8	34.2
Epinephrin solution A^{a}	79	1	1	200,000	5.19	153.4	188.6	35.2
Adnephrin solution B^{d}	80	1	1	100,000	5.20	153.2	187.4	34.2
Epinephrin solution A^{a}	81	1	1	200,000	5.21	152.2	186.4	34.2
Adnephrin solution B^{d}	82	1	1	100,000	5.22	152.8	187.4	34.6

B.

[Blood pressure experiment 6. Young female hound, weight 13.4 kilograms, subcutaneous injection morphine sulphate 135 milligrams. Balance of legend same as that of experiment 1.]

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Curare	98	1	1	....	5.6	....	....	....
Epinephrin solution A^{d}	111	1	1	100,000	5.46	100.8	119.8	19.0
Adnephrin solution B^{d}	112	1	1	50,000	5.48	98.6	118.6	20.0
Epinephrin solution A^{e}	123	1	1	100,000	6.25	90.2	112.8	22.6
Adnephrin solution B^{d}	124	1	1	50,000	6.27	92.2	114.4	22.2
Epinephrin solution A^{e}	125	1	1	100,000	6.29	94.6	115.0	20.4
Epinephrin solution A^{e}	113	1	1	100,000	5.49	98.0	121.8	23.8
Adnephrin solution B^{d}	114	1	1	50,000	5.51	98.6	121.0	22.4

Table II shows that the samples of adrenalin tested compare very favorably with fresh solutions of the standard solution A. Its keeping qualities are, however, not equal to some of the other preparations tested. As will be seen in Table XIV, this is evidenced by the loss of not a little of its vaso-constrictor action and also by the appearance of considerable color after having been diluted to one-tenth of its labeled strength.

TABLE II.—*Showing rises of blood pressure following intravenous injections of adrenalin and epinephrin.*

A.

[Blood-pressure experiment No. 1. See legend to Table I.]

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	49	½			4.13			
Adrenalin solution C^d	87	1	1	100,000	5.30	153.0	192.0	39.0
Epinephrin solution A^a	88	1	1	100,000	5.31	154.4	193.2	38.8
Adrenalin solution C^d	89	1	1	100,000	5.32	153.4	191.6	37.2
Epinephrin solution A^a	90	1	1	100,000	5.34	151.0	188.0	37.0
Adrenalin solution C^d	93	1	1	100,000	5.38	153.4	188.4	35.0
Epinephrin solution A^a	94	1	1	100,000	5.39	152.0	188.0	36.0
Adrenalin solution C^d	95	1	1	100,000	5.40	153.6	188.2	34.6
Epinephrin solution A^a	96	1	1	100,000	5.41	156.0	190.6	34.6

B.

[Blood-pressure experiment No. 7. Old hound, weight 30 kilograms, subcutaneous injection of 250 milligrams morphine sulphate. Balance of legend the same as that to Table I.]

Curare	17	1			12.34			
Adrenalin C^d	70	1	1	50,000	2.54	49.8	70.6	20.8
Epinephrin A^f	71	1	1	50,000	2.55	41.8	61.4	19.6

Table III shows that the fresh solutions of adrin tested should be labeled 1:1,000 instead of 1:500, since it requires 1 c. c. of a 1:50,000 solution of adrin to cause the same rise of blood pressure as does 1 c. c. of a 1:100,000 solution of epinephrin base. Judging from Table XIV it would seem that the color changes of this solution are not a good criterion by which to gauge the degree of deterioration undergone in a given solution. The usual characteristic red-orange color of the slightly decomposed solution of pure epinephrin is so faint that one accustomed to the color changes of ordinary epinephrin dissolved in acidulated saline might judge the solution to be of normal activity, whereas physiological assay would show the solution to be worthless. This is brought out clearly in Table XIV. Here it is shown that a solution one-tenth the labeled concentration, if allowed to stand 10 days, develops only a moderate depth of color, whereas it so loses in physiological activity that 0.5 c. c. no longer causes a rise of blood pressure.

TABLE III. *Showing rises of blood pressure following intravenous injections of adrin[a] and epinephrin.*

[Blood-pressure experiment No. 1. See legend to Table I.]

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	49	½	1	....	4.13	....	....	....
Epinephrin solution A[a]	103	1	1	100,000	5.54	162.0	209.4	47.4
Adrin solution D[d]	104	1	1	50,000	5.57	156.6	203.0	46.4
Adrin solution D[d]	108	1	1	50,000	6.6	168.6	208.4	39.8
Epinephrin solution A[a]	109	1	1	100,000	6.8	170.0	211.6	41.6
Adrin solution D[d]	110	1	1	50,000	6.10	163.4	203.8	40.4
Epinephrin solution A[a]	115	1	1	100,000	6.19	167.2	209.0	41.8
Adrin solution D[d]	116	1	1	50,000	6.21	168.2	210.0	41.8
Adrin solution D[d]	124	1	1	50,000	6.35	169.4	205.4	36.0
Epinephrin solution A[a]	125	1	1	100,000	6.38	172.6	209.2	36.6
Curare	126	½	....	....	6.43	....	....	....

[a] This solution is labeled 1:500.

Table IV shows that this preparation compares very favorably with the standard preparation. Its keeping properties are also very good, for, judging from Table XIV, the change in color and in physiological activity of a 1:10,000 solution allowed to stand 10 days is but slight.

TABLE IV. *Showing rises of blood pressure following intravenous injections of supracapsulin and epinephrin.*

[Blood pressure experiment No. 6, October 15, 1909. Young female hound, weight 13.4 kilograms, subcutaneous injection of 1.5 milligrams morphine sulphate. Balance of legend same as that of Table I, A.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*A. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	4	½	....	....	11.53	....	....	....
					P. m.	*P. m.*	*P. m.*	*P. m.*
Epinephrin solution A[d]	9	1	1	100,000	12.19	124.2	150.4	26.2
Supracapsulin solution F[d]	10	1	1	100,000	12.21	121.2	149.6	28.4
Epinephrin solution A[d]	11	1	1	100,000	12.23	121.8	149.6	27.8
Supracapsulin solution F[d]	12	1	....	100,000	12.25	120.4	149.0	28.6
Epinephrin solution A[d]	13	1	1	100,000	12.29	124.2	151.6	27.4
Supracapsulin solution F[d]	14	1	1	100,000	12.31	121.6	147.8	26.2
Epinephrin solution A[d]	15	1	1	100,000	12.32	122.0	150.0	28.0
Supracapsulin solution F[d]	16	1	1	100,000	12.37	126.0	150.0	24.0
Epinephrin solution A[d]	17	1	1	100,000	12.39	124.4	149.6	25.2

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Supracapsulin solution F[d]	71	1	1	50,000	3.54	160.4	185.8	25.4
Epinephrin solution A[d]	72	1	1	50,000	3.56	158.8	184.6	26.6
Supracapsulin solution F[d]	73	1	1	50,000	3.58	158.4	185.6	27.2
Epinephrin solution A[d]	84	1	1	50,000	4.21	148.6	173.6	25.0
Supracapsulin solution F[d]	85	1	1	50,000	4.23	149.8	174.4	24.6
Epinephrin solution A[d]	86	1	1	50,000	4.25	154.0	176.2	22.2
Supracapsulin solution F[d]	87	1	1	50,000	4.27	143.8	166.0	22.6
Supracapsulin solution F[d]	79	1	1	50,000	4.11	157.0	181.6	24.6
Epinephrin solution A[d]	80	1	1	50,000	4.13	159.2	183.6	24.4
Supracapsulin solution F[d]	81	1	1	50,000	4.15	155.6	182.4	26.8

Table V gives results which indicate that the samples of suprarenalin solution tested are less active than may be inferred from the concentration indicated on the label. Part A shows that 1 c. c. injections of a 1:50,000 solution of suprarenalin causes the same rise of blood pressure as does a similar injection of a 1:75,000 solution of epinephrin. In part B a 1:25,000 solution is the equivalent of a 1:35,000 solution of epinephrin. Judging from the data of Table XIV the keeping qualities of this solution are fairly good, for after standing ten days the diluted solution loses but little of its original physiological activity.

Table V.—*Showing rises of blood pressure following intravenous injections of suprarenalin and of epinephrin.*

[Blood pressure experiment No. 3, September 13, 1909. Young male dog, weight 9.3 kilograms, subcutaneous injection of 93 milligrams of morphine sulphate. Balance of the legend same as that of Table I, A.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Epinephrin solution A^{b}	150	1	1	75,000	6.44	127.6	170.8	43.2
Suprarenalin solution G^{d}	151	1	1	50,000	6.46	127.4	169.0	41.6
Epinephrin solution A^{b}	152	1	1	75,000	6.50	135.6	177.4	41.8
Epinephrin solution A^{b}	153	1	1	75,000	6.52	134.0	177.0	43.0
Suprarenalin solution G^{d}	154	1	1	50,000	6.57	140.8	184.4	43.6
Suprarenalin solution G^{d}	179	1	1	50,000	....	152.4	195.8	43.4
Curare	180	½	....	....	....	....	....	....
Epinephrin solution A^{b}	181	1	1	70,000	....	138.4	180.4	42.0

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Curare	139	½	....	....	6.8	....	....	....
Suprarenalin solution G^{d}	163	1	1	25,000	7.48	141.4	199.4	58.0
Epinephrin solution A^{b}	164	1	1	35,000	7.50	136.8	195.4	58.6
Suprarenalin solution G^{d}	165	1	1	25,000	7.53	138.2	197.6	59.4
Epinephrin solution A^{b}	167	1	1	35,000	8.1	150.0	205.2	55.2
Suprarenalin solution G^{d}	168	1	1	25,000	8.3	158.6	214.2	55.6
Suprarenalin solution G^{d}	174	1	1	25,000	8.16	144.0	194.4	50.4
Epinephrin solution A^{b}	175	1	1	35,000	8.18	148.2	200.0	51.8

Table VI.—A and B contain data that shows synthetic suprarenin solution to be only one-half as active as epinephrin solution. When 1 c. c. injections are made it is necessary in order to secure like rises of blood pressure to employ a 1:50,000 solution of synthetic suprarenin against a 1:100,000 solution of epinephrin base or some similar ratio as 1:25,000 against a 1:50,000. So it is well to bear in mind that this commercial solution as now made up is only one-half as active as 1:1,000 solutions of the natural epinephrin base.

The keeping qualities of solutions of synthetic suprarenin are apparently very good when kept properly sealed in the original package, but when diluted and kept under the conditions described in connection with Table XIII it is very much inferior to the best solutions of ephinephrin. This is shown both by the rapid change in color and by an appreciable loss in physiological activity.

TABLE VI. *Showing rises of blood pressure following intravenous injections synthetic suprarenin and of epinephrin.*

[Blood pressure experiment No. 3. See legend to Table V, A.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Suprarenin, synthetic II^d....	44	1	1	50,000	1.43	128.4	158.8	30.4
Epinephrin A^b..............	45	1	1	100,000	1.45	127.4	158.8	30.6
Epinephrin A^b..............	113	1	1	100,000	5.12	144.2	171.6	27.4
Suprarenin, synthetic II^d....	114	1	1	50,000	5.14	140.2	169.4	29.2
Epinephrin A^b..............	115	1	1	100,000	5.16	140.0	167.4	27.4
Suprarenin, synthetic II^d....	116	1	1	50,000	5.18	146.4	174.0	27.6
Epinephrin A^b..............	119	1	1	100,000	5.24	144.6	173.8	29.2
Suprarenin, synthetic II^d....	120	1	1	50,000	5.26	143.4	172.2	28.8
Epinephrin A^b..............	121	1	1	100,000	5.28	140.8	171.8	31.0
Suprarenin, synthetic II^d....	122	1	1	50,000	5.30	144.6	177.0	32.4
Epinephrin A^b..............	128	1	1	100,000	5.41	154.8	188.6	33.8
Suprarenin, synthetic II^d....	129	1	1	50,000	5.43	146.4	180.2	33.8

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Suprarenin, synthetic II^d....	132	1	1	25,000	4.49	148.8	196.6	57.8
Epinephrin A^b..............	133	1	1	50,000	4.55	140.0	196.4	56.4
Suprarenin, synthetic II^d....	134	1	1	25,000	4.57	134.8	189.0	54.2

B. SERIES *M* BOUGHT UPON THE OPEN MARKET FROM A RELIABLE PHARMACIST.

Table VII, A and B, show that the samples of adnephrin tested are only about one-half as active as an epinephrin solution of the same concentration (as given on the label). The keeping property of this solution is about the same as that of B^d described in Tables I and XIV.

Table VII.—*Showing rises of blood pressure following intravenous injections of adnephrin and of epinephrin.*

[Blood pressure experiment No. 4, September 15, 1909. Young female dog, weight 9 kilograms, subcutaneous injection of 90 milligrams of morphine sulphate. Balance of legend the same as that to Table I, A.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	7	½	......	......	12.32	......	......	......
Adnephrin B^{m}	20	1	1	50,000	12.59	144.6	169.8	25.2
Epinephrin A^{c}	21	1	1	100,000	1.2	140.0	163.6	23.6
Adnephrin B^{m}	22	1	1	50,000	1.4	140.8	166.8	26.0
Epinephrin A^{c}	23	1	1	100.000	1.6	141.8	167.6	25.8
Epinephrin A^{c}	25	1	1	100.000	1.10	136.2	163.8	27.6
Adnephrin B^{m}	26	1	1	50,000	1.12	136.4	164.0	27.6
Epinephrin A^{c}	27	1	1	100,000	1.14	139.4	168.2	28.8
Epinephrin A^{c}	33	1	1	100,000	1.30	140.2	164.0	23.8
Adnephrin B^{m}	34	1	1	50,000	1.32	141.0	166.6	25.6

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Curare	59^{a}	½	......	......	2.50	......	......	......
Epinephrin A^{c}	72	1	1	55,000	3.20	151.4	225.6	74.2
Adnephrin B^{m}	73	1	1	25,000	3.22	147.8	221.0	73.2
Curare	79	1	......	......	3.34	......	......	......
Epinephrin A^{c}	82	1	1	50,000	3.48	133.4	208.0	74.6
Adnephrin B^{m}	83	1	1	25,000	3.50	128.8	202.8	74.0
Epinephrin A^{c}	84	1	1	50,000	3.53	144.2	197.8	53.6
Adnephrin B^{m}	85	1	1	25,000	3.55	147.4	201.4	54.0

Table VIII shows about the same results as were described in connection with Table II. That is, the solution tested gives the same rise of blood pressure as does the standard epinephrin solution. Judging from the results given in Table XIV, this particular sample has, however, inferior keeping qualities to that described in connection with Table II.

TABLE VIII.—*Showing rises of blood pressure following intravenous injections of adrenalin and epinephrin.*

[Blood pressure experiment No. 4. See legend to Table VII.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c*	*Gram.*	*C. c.*	*P. m.*	*M. m*	*M. m.*	*M. m.*
Curare	164	1	....	....	8. 55	....	....	....
Adrenalin C^{m}	166	1	1	50,000	9. 4	154. 2	221. 4	67. 2
Epinephrin A^{c}	167	1	1	50,000	9. 6	158. 2	224. 4	66. 2
Adrenalin C^{m}	168	1	1	50,000	9. 8	161. 8	220. 2	58. 4
Epinephrin A^{c}	169	1	1	50,000	9. 11	157. 0	216. 2	59. 2
Adrenalin C^{m}	170	1	1	50,000	9. 13	158. 0	218. 0	60. 0
Epinephrin A^{c}	171	1	1	50,000	9. 15	151. 0	218. 0	68. 0
Adrenalin C^{m}	172	1	1	50,000	9. 17	154. 0	221. 2	67. 2

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Adrenalin C^{m}	178	1	1	25,000	9. 34	154. 8	254. 4	99. 6
Epinephrin A^{c}	179	1	1	25,000	9. 36	153. 6	254. 0	100. 4
Adrenalin C^{m}	180	1	1	25,000	9. 38	150. 2	242. 8	92. 6
Epinephrin A^{c}	181	1	1	25,000	9. 40	152. 0	242. 0	90. 0
Adrenalin C^{m}	182	1	1	25,000	9. 42	147. 8	239. 4	91. 6
Epinephrin A^{c}	183	$\frac{8}{10}$	1	25,000	9. 44	154. 0	229. 0	75. 0
Adrenalin C^{m}	184	$\frac{8}{10}$	1	25,000	9. 46	155. 2	228. 4	73. 2
Epinephrin A^{c}	187	1	1	25,000	10. 00	152. 0	258. 0	106. 0
Adrenalin C^{m}	188	1	1	25,000	10. 2	150. 4	257. 6	107. 2

Table IX.—This adrin solution differs greatly from the one described in connection with Table III. From the data given in this table it would seem that the sample had greatly deteriorated, for in order to secure rises of blood pressure that could be used with any degree of certainty the original solution of adrin had to be used in a concentrated form. A 1 : 15,000 solution of adrin caused the same rise of blood pressure as does a 1 : 200,000 epinephrin solution (see part A, Table IX), or a 1 : 7,500 solution of this sample is the equivalent of a 1 : 200,000 epinephrin solution. It would seem that the chief cause of this deterioration is to be attributed to the method of preserving.

Table XIV shows that all the samples of adrin tested deteriorated rapidly when once diluted and at the time of buying this preparation upon the open market the first sample had to be returned on account of its bad appearance.

TABLE IX.—*Showing rises of blood pressure following intravenous injections of adrin and of epinephrin.*

[Blood pressure experiment No. 6. See legend to Table I, B.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Adrin D^m	89	1	1	15,000	4.32	140.6	147.4	6.8
Epinephrin A^e	90	1	1	400,000	4.34	136.4	141.8	5.4
Adrin D^m	91	1	1	15,000	4.35	134.4	139.8	5.4
Epinephrin A^e	92	1	1	400,000	4.36	138.0	143.2	5.2

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Curare	98	1			5.6			
Epinephrin A^e	99	1	1	200,000	5.9	88.0	97.8	9.8
Adrin D^m	100	1	1	7,500	5.10	91.0	100.0	9.0
Epinephrin A^e	102	1	1	200,000	5.11	93.8	102.4	8.6
Adrin D^m	103	1	1	7,500	5.12	95.4	102.6	7.2
Adrin D^m	105	1	1	7,500	5.16	99.8	106.0	7.2
Epinephrin A^e	106	1	1	200,000	5.17	99.8	108.6	8.8
Adrin D^m	107	1	1	7,500	5.18	102.6	107.6	5.5

The results given in Table X show the sample tested to be only about one fourth as active as the standard epinephrin solution. It is evident that this preparation had deteriorated, for not only was the solution colored but there was present a granular precipitate. (See Table XIV.) Judging from the results recorded in Table XIV, the keeping property of this solution is not very good. Color, as observed without the tintometer, in this case, is not a very safe index of the extent to which the solution has deteriorated, although the presence of considerable "neutral tint" in all probability more than makes up for the lack of red and yellow color measured with the tintometer. Furthermore, when the active substance begins to precipitate out the color changes may come to a standstill or gradually decrease in degree.

TABLE X.—*Showing rises of blood pressure following intravenous injections of caprenalin and epinephrin.*

[Blood pressure experiment No. 7. See legend to Table II.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	17	1	....	....	12.34	....	....	....
Epinephrin A^{f}	37	1	1	40,000	1.24	80.6	111.0	30.4
Caprenalin E^{m}	38	1	1	10,000	1.27	78.0	108.6	30.6

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Caprenalin E^{m}	43	1	1	20,000	1.36	62.0	78.2	16.2
Epinephrin A^{f}	44	1	1	80,000	1.39	63.6	80.4	16.8
Caprenalin E^{m}	45	1	1	20,000	1.41	61.0	77.2	16.2
Epinephrin A^{f}	48	1	1	80,000	1.48	60.0	76.8	16.8
Caprenalin E^{m}	49	1	1	20,000	1.50	55.0	70.8	15.8

Table XI shows that this sample of supracapsulin is about the equal of that described in connection with Table IV. Judging from the results given in Table XIV, its keeping properties are very good.

TABLE XI.—*Showing rises of blood pressure following intravenous injections of supracapsulin and of epinephrin.*

[Blood pressure experiment No. 4. See legend to Table VII.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	79	1			3.34	....	....	....
Supracapsulin F^{a}	92	1	1	50,000	4.32	158.2	210.4	52.2
Epinephrin A^{c}	93	1	1	50,000	4.34	154.2	207.0	52.8
Supracapsulin F^{a}	94	1	1	50,000	4.36	153.4	206.8	53.4
Epinephrin A^{c}	95	1	1	50,000	4.38	153.4	207.4	54.0
Supracapsulin F^{a}	96	1	1	50,000	4.39	153.4	206.4	53.0
Curare	100	1		Weak.	4.47	....	....	....
Supracapsulin F^{a}	111	1	1	50,000	5.17	150.2	205.8	55.6
Epinephrin A^{c}	112	1	1	50,000	5.19	154.0	208.0	54.0
Supracapsulin F^{a}	113	1	1	50,000	5.21	154.2	208.2	54.0

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Curare	100	1			......	....	....	....
Epinephrin A^{c}	116	1	1	25,000	5.33	157.0	247.0	90.0
Supracapsulin F^{a}	117	1	1	25,000	5.35	148.8	239.8	91.0
Epinephrin A^{c}	118	1	1	25,000	5.37	145.4	246.4	101.0
Supracapsulin F^{a}	119	1	1	25,000	5.40	133.0	234.2	101.2
Epinephrin A^{c}	120	1	1	25,000	5.42	131.4	232.0	100.6
Supracapsulin F^{a}	121	1	1	25,000	5.44	130.8	230.0	99.2

The results of Table XII show that the suprarenalin sample bought on the open market is about the same as that described in connection with Table V. A 1:75,000 and a 1:37,500 solution of epinephrin solution causes the same rise of blood pressure as does a 1:50,000 and a 1:25,000 solution, respectively, of suprarenalin. The keeping properties of this solution are about the same as that described for series *d*. (See Table V.)

TABLE XII.—*Showing rises of blood pressure following intravenous injections of suprarenalin and of epinephrin.*

[Blood pressure experiment No. 4. See legend to Table VII.]

A.

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	134	1	....	Weak.	7.3	....	....	....
Suprarenalin G^m	135	1	1	50,000	7.6	168.4	206.6	38.2
Epinephrin A^c	136	1	1	75,000	7.8	164.2	203.8	39.6
Suprarenalin G^m	140	1	1	50,000	7.16	160.0	200.4	40.4
Epinephrin A^c	141	1	1	75,000	7.18	159.8	200.8	41.0

B.

Solution injected.	Injection.	Amount injected.	Base.	Normal saline.	Time of injection.	Before.	After.	Rise.
Epinephrin A^c	146	1	1	37,500	7.35	141.4	220.4	79.0
Suprarenalin G^m	147	1	1	25,000	7.37	144.2	224.0	79.8
Epinephrin A^c	148	1	1	37,500	7.39	140.2	220.2	80.0
Suprarenalin G^m	151	1	1	25,000	7.45	146.2	215.8	69.6
Curare	151	1	....	Weak.	7.46	....	....	....
Epinephrin A^c	152	1	1	37,500	7.48	160.8	230.4	69.6
Suprarenalin G^m	158	1	1	25,000	8.12	155.4	235.6	80.2
Epinephrin A^c	169	1	1	37,500	8.13	156.2	235.8	79.6
Suprarenalin G^m	160	1	1	25,000	8.15	153.2	232.2	79.0
Epinephrin A^c	161	1	1	37,500	8.18	151.6	227.2	75.6
Suprarenalin G^m	162	1	1	25,000	8.20	153.2	228.4	75.2

The results given in Table XIII show that this sample of synthetic suprarenin when used fresh has about the same activity as that described in connection with Table VI. The keeping properties of this solution, according to the data of Table XIV, seem to be about the same as that in series *d*. When exposed to light, as were samples H^{d3} and H^{m3}, the one belonging to the *m* series kept the better of the two, though both deteriorated much more rapidly than did the solutions H^{d2} and H^{m2}.

Table XIII.—*Showing the rises of blood pressure caused by intravenous injections of synthetic suprarenin and of epinephrin.*

A.

[Blood pressure experiment No. 6. See legend to Table I, B.]

Solution injected.	Injection.	Amount injected.	Concentration of solution.		Time of injection.	Blood pressure, measured by a mercury manometer.		
			Base.	Normal saline.		Before.	After.	Rise.
	No.	*C. c.*	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Curare	57	0.8	....	....	....	....	....	....
Epinephrin A^e	60	1	1	50,000	3.26	165.8	192.0	26.2
Suprarenin synthetic H^m	61	1	1	25,000	3.28	170.6	197.6	27.0
Epinephrin A^e	62	1	1	50,000	3.30	171.8	199.8	28.0
Suprarenin synthetic H^m	63	1	1	25,000	3.32	175.6	202.6	27.0
Suprarenin synthetic H^m	68	1	1	25,000	3.45	157.6	184.4	26.8
Epinephrin A^e	69	1	1	50,000	3.47	156.0	184.4	28.4

B.

[Blood pressure experiment No. 7. See legend to Table II.]

Curare	17	1	....	....	12.34	....	....	....
Epinephrin A^f	19	1	1	100,000	12.37	83.0	100.0	17.0
Suprarenin synthetic H^m	20	1	1	50,000	12.39	83.8	99.6	15.8
Suprarenin synthetic H^m	25	1	1	25,000	12.53	91.0	111.8	20.8
Epinephrin A^f	26	1	1	50,000	12.56	104.4	125.4	21.0
Epinephrin A^f	28	1	1	50,000	1.1	78.6	106.6	28.0
Suprarenin synthetic H^m	29	1	1	25,000	1.3	82.4	109.4	27.0
Suprarenin synthetic H^m	31	1	1	25,000	1.7	89.6	111.2	21.6
Epinephrin A^f	32	1	1	50,000	1.11	84.4	106.0	21.6

TINTOMETER MEASUREMENTS OF THE DIFFERENT SHADES OF COLOR OBSERVED IN COMMERCIAL SAMPLES OF EPINEPHRIN.

In testing commercial solutions of epinephrin the method of expressing the amount of color in units of the ordinary colorimeter is unsatisfactory; for not only do the solutions develop different degrees of color depth, but more than one color tint is involved. This introduction of two or more tints requires the use of some measuring device that admits of indicating the exact color and reproducing the visual sensation excited by the solutions in question. Lovibond's tintometer meets these requirements admirably and was used in making readings recorded in Tables XIV and XV. The present section gives only approximately an idea of the relation existing between the color of a commercial solution and its physiological activity. Here we are concerned only with such relative values as aid in conveying a general idea of the condition of the solution tested, and hence it is left for a subsequent paper to discuss more in detail the relation existing between the different shades of color developed in pure solutions of epinephrin and the respective physiological activity.

It is well known that epinephrin solutions, when thus diluted and kept in half-filled containers, deteriorate, as a rule, quite rapidly even in a dark place; and if kept in a well-lighted room in colorless containers they lose even more quickly their physiological activity. It is also well known that different commercial samples vary greatly in their keeping properties. A more careful examination into this phase of the subject seemed well worth while, and to this end some preliminary experiments have been made, the results of which are summarized in Tables XIV and XV.

TABLE XIV.—*Showing changes in color and in physiological activity undergone, in the course of ten days, by 1:10,000 solutions of commercial epinephrin solutions.*

Laboratory number of solution.	Commercial name of solution.	Oct. 19.		Oct. 20.		Oct. 21.		Oct. 22.		Oct. 23.	
		Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Red.	Yellow.
B^{b2}	Adnephrin	0.02	0.03	0.02	0.03	0.04	0.05	0.06	0.08	0.06	0.08
B^{m2}	Adnephrin	.02	.03	.02	.03	.04	.05	.06	.08	.06	.08
C^{d2}	Adrenalin	.05	.04	.14	.15	.40	.30	.80	.40	1.30	.78
C^{m2}	Adrenalin	.12	.11	.45	.27	1.20	.70	2.10	1.10	3.10	1.50
D^{d2}	Adrin 1:500	.07	.08	.15	.14	.41	.30	.70	.60	1.00	.80
D^{m2}	Adrin 1:1,000	1.25	1.60	1.32	1.95	1.40	2.10	1.06	2.20	1.15	2.30
								Dark.	Ppt.	Blue.	.90
E^{m2}	Caprenalin	.50	.40	.80	.60	1.00	.70	1.08	.72	1.00	.72
F^{d2}	Supracapsulin	.05	.03	.05	.03	.06	.07	.25	.10	.44	.18
F^{m2}	Supracapsulin	.10	.04	.13	.05	.20	.26	.35	.26	.49	.18
G^{d2}	Suprarenalin	.05	.03	.04	.03	.11	.07	.34	.20	.85	.55
G^{m2}	Suprarenalin	.17	.27	.17	.20	.40	.30	.50	.40	.80	.50
H^{d2}	Suprarenin synthetic	.05	.06	.10	.08	1.10	.50	2.30	.70	3.20	1.30
H^{m2}	Suprarenin synthetic	.11	.10	.17	.13	.89	.40	1.38	.58	2.40	1.10
H^{d3}	Suprarenin synthetic	......	......	.05	.06	......	......	1.50	.50	4.60	2.00
H^{m3}	Suprarenin synthetic	......	......	.11	.10	......	......	.50	.10	.80	.50

Laboratory number of solution.	Commercial name of solution.	Oct. 24.		Oct. 25.		Oct. 27.		Oct. 29.		Rise of blood pressure caused by 1 c.c. injections of a 1:10,000 solution.	
		Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Oct. 29. (fresh).[1]	Oct. 29. (old).[2]
										Mm.	*Mm.*
B^{b2}	Adnephrin	0.06	0.08	0.08	0.13	0.23	0.23	0.32	0.32	74.2	69.2
B^{m2}	Adnephrin	.06	.08	.08	.13	.20	.22	.25	.27	67.8	58.0
C^{d2}	Adrenalin	2.10	1.00	2.20	1.10	3.20	1.34	5.60	2.60	106.0	75.0
C^{m2}	Adrenalin	4.30	2.00	4.40	2.50	7.00	3.50	8.40	4.50	66.6	64.4
D^{d2}	Adrin 1:500	1.00	1.00	1.03	1.02	1.05	1.05	1.05	1.05	0.0	0.0
D^{m2}	Adrin 1:1,000	1.30	2.70	1.45	3.20	1.95	4.02	2.45	4.40	0.0	0.0
		Blue.	1.20	Blue.	1.20	Blue.	1.20	Blue.	1.20		
E^{m2}	Caprenalin	1.00	.72	1.00	.72	1.00	.72	.80	.72	0.0	0.0
F^{d2}	Supracapsulin	.60	.40	.70	.44	.80	.54	1.10	.64	102.6	101.6
F^{m2}	Supracapsulin	.70	.35	.75	.40	.86	.50	1.25	.64	99.0	93.4
G^{d2}	Suprarenalin	1.10	.50	1.32	.58	1.75	.90	2.25	1.05	97.6	78.6
G^{m2}	Suprarenalin	1.00	.70	1.10	.77	1.35	.80	1.70	1.00	83.0	79.0
H^{d2}	Suprarenin synthetic	3.80	1.40	3.80	1.40	5.00	2.10	6.00	2.50	74.2	60.0
H^{m2}	Suprarenin synthetic	3.00	1.20	3.20	1.20	4.60	2.00	6.00	2.50	73.8	60.2
H^{d3}	Suprarenin synthetic	5.30	2.50	5.70	2.50	8.00	3.40	8.50	4.00	74.2	24.4
H^{m3}	Suprarenin synthetic	1.10	.60	1.20	.62	2.20	.80	2.90	1.10	73.8	48.0

(1) Solution made Oct. 29.

(2) Solution made Oct. 19.

Table XIV illustrates the changes in color undergone by the various commercial solutions of epinephrin already discussed, they having been diluted with normal saline solution. These solutions were made by measuring out 2 c. c. of the original 1:1,000 solution, diluting this to 1:10,000, and preserving it in a ground glass-stoppered bottle made of the best German flint glass. The bottles, in each case being about two-thirds full, were kept in the dark except when the tintometer readings were made. On the tenth day after making up the 1:10,000 solutions their physiological activity was compared with a freshly diluted solution (1:10,000 from same original package) by intravenous injection of 0.5 c. c. of the old and new solutions into the right and left saphenous vein, respectively, of a dog and measuring the resulting rise of blood pressure.

It will be seen that unless certain factors enter in to alter or retard the normal color that usually develops in an epinephrin solution upon standing, the strength of the solution is roughly in inverse proportion to the degree of color until the decomposed material begins to precipitate out. If, however, such substances as sulphites and deoxidizing agents be present then a solution may register but little color, and at the same time possess but little vaso-constrictor action. Color then is not always a safe criterion by which to judge of the value of a solution, but the presence of a precipitate ought always to suggest to the pharmacist that the solution should be tested physiologically before being dispensed, even though the label says it had been tested before storing in containers at the laboratory.

SUMMARY AND CONCLUSIONS.

Of the seven different brands of epinephrin examined only three possessed an activity that equaled the standard. The other solutions varied anywhere from 3.75 to 71 per cent of the required activity. (See Table XV.) As is brought out in a more graphic manner in this table, some of the solutions were, from the physician's point of view, worthless, and perhaps even dangerous. Certain solutions, though showing a high degree of activity upon opening the original package, quickly deteriorated in spite of the extra precautions taken to guard against conditions known to further this process. On the other hand, some of the preparations now upon the market made by American concerns are of the very highest quality.

TABLE XV.—*Tintometer determinations of color observed and the relative physiological activity of the original 1:1,000 solutions.*

Laboratory number of solution.	Commercial name of solution.	Manufacturer's serial number.	Color of solution.	Proportion of—		Precipitate present.	Relative physiologic activity expressed in per cent of A.
				Red.	Yellow.		
A (1)	Epinephrin		Red-orange	0.10	0.05	None	(4) 100.00
B^{d} (2)	Adnephrin	60802 FR	Yellow-orange	.20	.40	None	50.00
B^{m} (3)	Adnephrin	62912 ER	Yellow-orange	.20	.40	None	50.00
C^{d}	Adrenalin	1360016	Yellow-orange	.52	.70	None	100.000
C^{m}	Adrenalin	1335177	Yellow-orange	.90	1.10	None	100.00
D^{d}	Adrin	A 76797	Yellow-orange	.22	.72	None	50.00
D^{m}	Adrin	102009	Yellow-orange	5.58	12.70	Abundant.	3.75
E^{m}	Caprenalin	1302244	Red-orange	2.10	2.00	Abundant.	25.00
F^{d}	Supracapsulin		Yellow-orange	.12	.16	None	100.00
F^{m}	Supracapsulin		Red-orange	.25	.24	Trace	100.000
G^{d}	Suprarenalin	670074	Yellow-orange	.15	.16	None	66 to 71
G^{m}	Suprarenalin	670065	Yellow-orange	.82	.84	None	66.66
H^{d}	Suprarenin synthetic.	SA 2306 F 13452	Red-orange	.24	.21	Trace	50.00
H^{m}	Suprarenin synthetic.	IH 2209 T 57630	Orange	.50	.50	Some	50.00

(1) Solution A made up on morning of experiment, from a pure sample of natural base, the optical activity f which is $[\alpha]_{D}^{26.4^\circ} = -53.40^\circ$.

(2) Series "d" bought directly from manufacturers or from their agents.

(3) Series "m" bought on the open market from a reliable druggist.

(4) The figures to the right of the decimal point are given simply because the calculations come out thus, nd not because the fractional parts have any practical value.

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TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN No. 62

August, 1910

THE TAXONOMIC VALUE OF THE MICROSCOPIC STRUCTURE OF THE STIGMAL PLATES IN THE TICK GENUS DERMACENTOR

By

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SUMMARY.

The specific determination of ticks is attended in many cases with considerable difficulty. In view of the importance of these giant mites as transmitters of disease, every newly recognized character which can be utilized in classification is of value. In the present paper the microscopic structure of the stigmal plates is shown to be a taxonomic character of considerable importance in the genus *Dermacentor*.

The species of *Dermacentor* which has been shown experimentally by King and Ricketts to act as transmitter of Rocky Mountain spotted fever in Montana is *Dermacentor andersoni*.

The tick which Salmon and Stiles (1901) considered identical with *Dermacentor reticulatus* of Europe is shown to represent a new species, *Dermacentor salmoni*.

For bibliographic references, see Stiles & Hassall, Index Catalogue of Medical and Veterinary Zoology <Bull. 39, U. S. Bureau of Animal Industry.

THE TAXONOMIC VALUE OF THE MICROSCOPIC STRUCTURE OF THE STIGMAL PLATES IN THE TICK GENUS DERMACENTOR.[a]

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INTRODUCTION.

To find characters which may be readily used in distinguishing genera and species is always an interesting study for the systematist. When the group of animals in question may act as transmitters or as cause of disease, this systematic study becomes especially important, both theoretically and practically.

Since it has been shown that certain species of ticks act as transmitters of certain diseases, this group of arachnoids has been subjected to more careful study than heretofore and numerous new species have been described. To recognize these species, even when detailed diagnoses are given, as in the writings of Neumann especially, is not unattended with difficulty, and even the special student in this group is liable to fall into error in determining specimens. Every new character which can be shown to be of taxonomic value is, therefore, of practical importance at the present time, and its practical value is proportionate to its ease of application.

Salmon and Stiles (1901a, 447) indicated that the "punctation" of the stigmal plate presented a character of value in the genus *Dermacentor;* Neumann also has used this character to a slight extent, while Banks (1908), and Cooper and Robinson (1908) have adopted it in recent papers. Since the publication of the joint paper (1901a) by Salmon and Stiles, I have become more and more convinced of the value of this character, not only in *Dermacentor* but also in some other genera. With this increased conviction a second conviction has developed, namely, that with a few exceptions the determination of the North American ticks by the usual method, namely, a study with the hand lens of an uncleared, unmounted specimen, is very likely to lead the observer to erroneous conclusions.

To give an illustration: Several years ago, I became convinced that the common tick of Montana was distinct from the form which

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[a] Manuscript submitted for publication December 21, 1909.

Salmon and Stiles looked upon as *Dermacentor reticulatus* (=*D. salmoni*); Neumann considered that *D. reticulatus* of Salmon and Stiles was in reality Marx's *D. occidentalis;* specimens of the Montana tick were sent to Neumann, with the interpretation that they represented a new species and with the request to compare the Montana form with Marx's types which were then in his possession; with his usual courtesy, Neumann made the comparison and wrote that he agreed that the Montana ticks represented a new species; later, Neumann returned our government collection of ticks to Washington; he also sent four specimens of the European *D. reticulatus;* in the meantime I had been studying the American species of *Dermacentor* from the standpoint of the microscopic structure of the stigmal plate, and becoming thoroughly convinced that the Montana tick was absolutely distinct from what I found in the collection labeled *D. reticulatus* and what I had always supposed to be that species, I used for the Montana form the manuscript name *D. andersoni.*

Upon receiving the material from Neumann, this was reexamined, especially with reference to the miscroscopic structure of the stigmal plate, and this restudy resulted in some very unpleasant surprises, for instance:

(1) Neumann's four European specimens (two males and two young females) of *D. reticulatus* agree fairly well with each other in the structure of their stigmal plates; these plates are of a type very similar to that found in the American species *D. electus* seu *variabilis.* Coxæ IV of the two males show very marked variations. The palpi of one male and of one female agree fairly well in outline, but are markedly different from the palpi of the other male and the other female, while the palpi of the latter pair are of rather uniform outline. Only one specimen (a male) shows a prominent retrograde prolongation on article 2 of the palpi. One pair is not especially difficult to distinguish from the American *D. electus;* the male and female of other pair are separated from *D. electus* with somewhat greater difficulty. It seems quite clear that the American *D. electus* and the European *D. reticulatus* are exceedingly closely related. Further, both species vary so in outline of the palpi and of coxæ IV that many systematists, working on single specimens, would recognize these variations as representing several distinct species.

(2) Marx's types of *D. occidentalis*, which Neumann considered as representing a variety of the European *D. reticulatus*, are radically different from this species; they are closely allied specifically to *D. andersoni* (with which for a time I considered them identical), but they are distinct from the American form which was considered as *D. reticulatus* and which Neuman identified with *D. occidentalis.*

(3) The American form which I have always viewed as *D. reticulatus* is totally distinct from Neumann's European material of this species.

In view of this experience, it was decided to determine ticks hereafter only when the specimens could be treated with caustic and mounted in such a way as to permit the use of medium power lenses (as 8 and 4 mm.) in studying them; further, it was found that by a careful study of the stigmal plate of mounted skins there is usually but little difficulty in determining the specimens (of *Dermacentor*, at least) upon this organ alone.

The purpose of the present paper is to show the value of the stigmal plate in dividing the North American species of *Dermacentor* into four groups and in differentiating the species. A preliminary communication on this subject was published in the Proceedings of the Entomological Society of Washington, volume 9 (1–4), 1908, pages 10–11.

TECHNIQUE.

The specimens are prepared as follows: A slit is made in the caudal end of the tick, usually with a sharp knife or scissors, and in such a way as not to cut the stigmal plates; the specimen is placed in a weak caustic solution (about 2 to 5 per cent) for 12 to 96 hours, according to the condition of the material; with sharp, pointed forceps, or with a small scalpel, the entire mass of soft tissue is removed through the caudal slit; the skin is then passed through water, the alcohols, and xylol, and mounted in balsam; for pressure, a pair of cover-glass forceps, such as are in common use in bacteriological work, or a lead weight the shape of an elongated bullet, is then placed on the preparation which is now dried on a radiator, or in an oven. It is wise to examine the skin in xylol before mounting it in balsam, and during this examination legs IV are so arranged as not to cover the stigmal plates; if necessary, legs IV are removed from the skin.

Superfamily IXODOIDEA[a] Banks, 1894.

It may be well to review the classification of ticks, before passing to the genus *Dermacentor*.

Ticks represent giant mites. They belong to the superfamily *Ixodoidea* of the order *Acarina*, class *Arachnida*.

Authors are, however, not yet in entire accord relative to the general classification of the group.

The superfamily *Ixodoidea* may be divided into two families, as follows:

Scutum absent; capitulum inferior in adult, terminal in hexapod larva; claws without pulvillum; palpi cylindrical; stigmal plate between legs III and IV; sexual dimorphism slight .... *Argasidæ*

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[a] For synonymy, see Salmon and Stiles, 1901a, pp. 383–384.

Scutum present; capitulum terminal in all stages; claws with pulvillum; articles 2 and 3 of palpi usually strongly excavate in long axis on median margin, article 4 small, subterminal of article 3; stigmal plate caudolateral of coxæ IV; sexual dimorphism very pronounced__________*Ixodidæ*

It is particularly the family *Ixodida* which interests us in the present paper.

Family IXODIDÆ[a] Murray, 1877.

FAMILY DIAGNOSIS.—*Ixodoidea* (p. 11): Scutum present. Capitulum terminal. Digit of mandibles provided with two articles; internal article with dorsal process; external article elongate, articulated with the internal article and bearing on its free border two to five teeth, which increase in size from the distal to the proximal. Palpi with articles 2 and 3 usually distinctly valvate in long axis on their median surface; article 4 very short in adult, as tactile appendage, situated in a ventro-terminal depression of article 3. Legs a little unequal, pair II shortest, pair IV longest; femur and tibia presenting pseudo-articulation near proximal end, except in pair I, where the pseudo-articulation is distal; tarsi with pulvillum. Stigmata dorso-caudad of coxæ IV. Genital pore ventro-median at height of coxæ I to III; a pair of sexual grooves extend from here caudad, more or less divergent, toward caudal margin. Sexual dimorphism very marked.

Male.—Usually smaller than female, flatter, often of less regularly oval contour, anterior pole being much narrower than posterior; dorsal shield covers entire dorsal surface or all but a marginal band; caudal margin ordinarily divided into eleven quadrangular festoons, distributed between the two stigmata, and often extending under the ventral surface. These represent in some cases marginal shields, the number and form of which are often variable.

Female.—At first flat, later may become very thick; dorsal shield confined to cephalic part of dorsum, remains comparatively small. Capitulum with two symmetrical porose areas, not found in larvæ, nymphs, or males.

TYPE-GENUS.—*Ixodes* Latreille, 1796.

This family may be divided in various ways, but authors are not yet entirely in accord relative to the details of the subdivisions.

Canestrini[b] (1890a, 491) divided the Italian genera of this family, then known to him, into three groups, as follows:

Ventral surface of male entirely covered with shields; genus *Ixodes*___*Poliopli.*
Ventral surface of male with four anal shields; genera *Hyalomma*, *Rhipicephalus*____________________*Tetraopli.*
Ventral surface of male without anal shields; genera *Amblyomma* (and *Aponomma*), *Dermacentor*, *Hæmaphysalis*____________________*Anopli.*

This division is a very easy one to make, provided one has male specimens, but as it is based upon a secondary sexual character it is not entirely free from objection.

Neumann (1901a, 323), who recognized *Ixodida* s. str. as a subfamily, divided the genera of this group as follows:

Rostrum long; genera *Ixodes*, *Eschatocephalus*, *Aponomma*, *Amblyomma*, *Hyalomma*____________________*Ixoda.*
Rostrum short; genera *Hæmaphysalis*, *Rhipicephalus*, *Dermacentor* *Rhipicephala.*

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[a] For synonymy, see Salmon and Stiles, 1901a, p. 414.

[b] Original paper not accessible at present. See Neumann, 1904a, p. 446.

Salmon and Stiles (1901a, 384) divided the *Ixodidæ* s. str. into two subfamilies, as follows:

Palpi short, subtriangular, not or only slightly longer than [the two together are] broad; capitulum short; cephalic margin of body emarginate for insertion of capitulum; genera *Rhipicephalus*, *Boophilus*, *Hæmaphysalis*, *Dermacentor*---*Rhiphicephalinæ*.

Palpi longer than broad; capitulum long; cephalic margin of body straight or emarginate; genera *Ixodes*, *Eschatocephalus*, *Aponomma*, *Amblyomma*, *Hyalomma* --*Ixodinæ*.

Since 1901 several new genera have been described.

Neumann (1904a, 447) has reverted to Canestrini's classification, with some change of names, as follows:

Ventral surface of male entirely covered with plates; anal groove surrounds anus anteriorly and is independent of genital grooves; eyes absent; rostrum elongate ---*Ixodeæ*.

Ventral surface of male with two adanal plates, usually accompanied by accessory plates; anal groove surrounds the anus caudally and usually joins, anteriorly, the genital grooves; eyes present; rostrum long or short. *Rhipicephaleæ*.

Ventral surface of males without adanal plates; anal groove surrounds anus caudally and usually joins, anteriorly, the genital grooves; eyes often present; rostrum long or short--------------------------------*Amblyommeæ*.

A later and rather extensive division of the genera in question is that proposed by Lahille (1905a, 21–23) who accepts *Ixodidæ* s. str. as a distinct family and divides the ticks as follows:

Suborder ARPAGOSTOMA [=superfamily *Ixodoidea*].

1. Capitulum inferior; scutum absent; claws without pulvillum; palpi cylindrical, nearly uniform; stigmal plates between legs III and IV; sexual dimorphism but little marked---------------------------------*Argasidæ*, 2.
   Capitulum terminal; scutum present; claws with pulvillum; palpal articles 2 and 3 excavate on median surface; stigmal plates caudally of legs IV; sexual dimorphism very pronounced --------------------*Ixodidæ*, 3.

ARGASIDÆ.

2. Tegument chagreened; hood does not project anteriorly of body; palpi not projecting anteriorly of body; lateral borders of body thin; ventral grooves very slightly marked; eyes absent; type *Acarus reflexus* Fabricius, 1794-------------------------------------*Argas* Latreille, 1795.
   Tegument mamillate (hemispherical elevations); hood projects anteriorly in form of a beak; palpi visible anteriorly, from above; lateral borders of body thick; ventral anal, preanal, and postanal grooves very marked; eyes present or absent; type *Argas savignyi* Audouin, 1827, *Ornithodoros* Koch, 1844.

IXODIDÆ (based on males).

3. Anal plates absent [compare *Amblyommea* Neumann, 1904]; eyes present or absent---------------------------------*Anopli* Canestrini, 4.
   Anal plates present in pairs (4–6) [compare *Tetraapoli* Canestrini, and *Rhipicephalea* Neumann, 1904]; eyes distinct (ommata)------*Artiopli*, 8.
   Anal plates present in uneven numbers (as 5) [compare *Poliopli* Canestrini and *Ixodea* Neumann, 1904]; eyes absent (anommata)----*Perissopli*, 10.

Anopli.[a]

4. Eyes absent (anommata)---------------------------------5.
   Eyes distinct (ommata)---------------------------------7.
5. Article 2 of palpi drawn out laterally into sharp points; type *H. concinna* Koch, 1844 ---------------------*Hæmaphysalis* Koch, 1844.
   Article 2 of palpi not drawn out laterally into sharp points--------------6.
6. Anal groove present; type *Ixodes gervaisi* Lucas, 1847,
   Aponomma Neumann, 1899.
   Anal groove absent; type *Ixodes transversalis* Lucas, 1844,
   Neumanniella Lahille.
7. Palpi longer than broad; type *Acarus cajennensis* Fabricius, 1787.
   Amblyomma Koch, 1844.
   Palpi scarcely longer than broad; coxæ IV very large; type *Acarus reticulatus* Fabricius, 1796-------------*Dermacentor* Koch, 1844.

Artiopli.

8. Palpi scarcely longer than broad; dorsal surface of base of capitulum ("prosoma") hexagonal-----------------------9.
   Palpi longer than broad; dorsal surface of base of capitulum rectangular; type *Acarus ægyptius* Linné, 1758---------*Hyalomma* Koch, 1844.
9. Stigmal plates circular; type *Ixodes annulatus* Say, 1821
   Boophilus Curtice, 1891.
   Stigmal plates comma-shaped; type *Ixodes sanguineus* Latreille, 1804
   Rhipicephalus Koch, 1844.

Perissopli.

10. Palpi elongate, canaliculate; type *Acarus ricinus* Linné, 1746 [1758]
    Ixodes Latreille, 1796.
    Palpi not canaliculate; type *E. gracilipes* Frauenfeld
    Eschatocephalus Frauenfeld, 1853.
    Palpi pyriform; (penultimate) article 3 conical; type *Hyalomma puta* Cambridge, 1879------------*Ceratixodes* Neumann, 1902.

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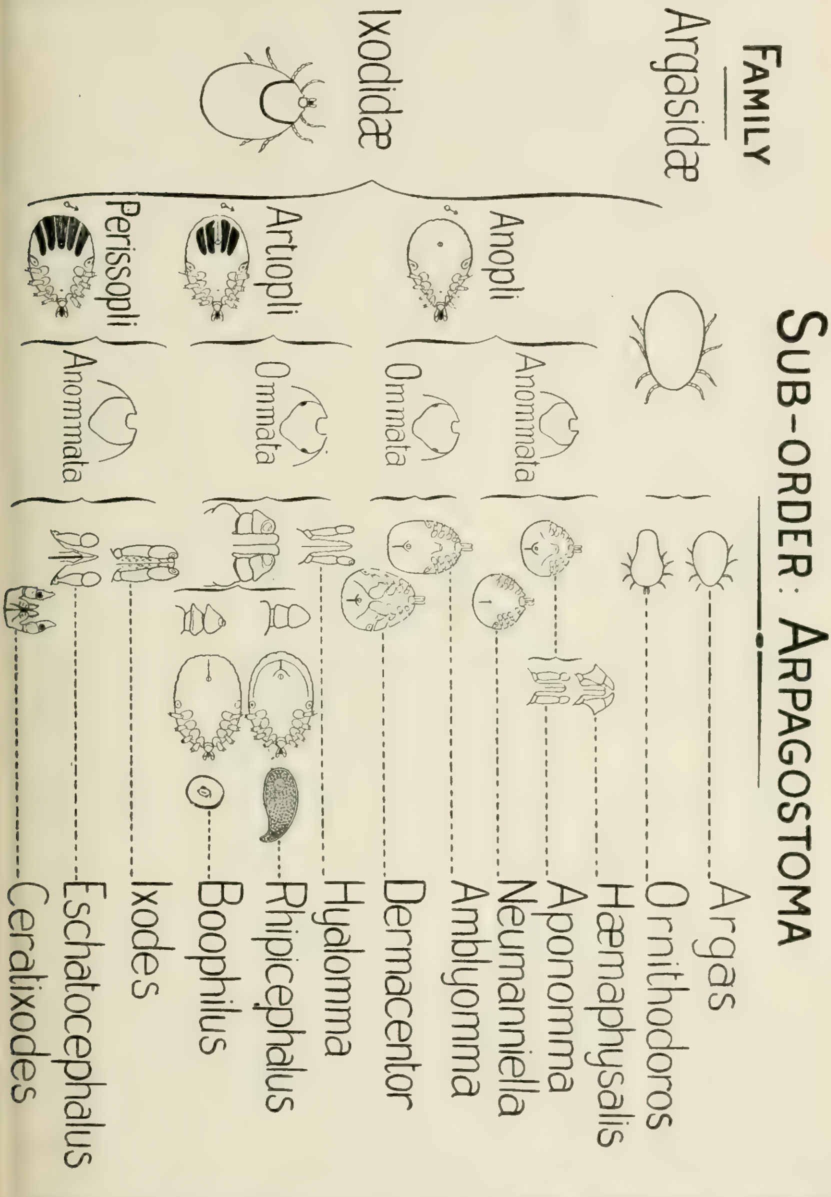
[a] Nuttall and Warburton (1908, 398) have recently established an additional genus which would fall into this group, namely:

Rhipicentor: Male resembles *Rhipicephalus* dorsally, *Dermacentor* ventrally. Basis capituli hexagonal with very prominent lateral angles. Coxa I strongly bifid and overlapping coxa II in male and female; male coxæ progressively increasing in size to pair IV, which is much the largest, pairs II–IV bifid. Adanal shields absent. Eyes present.

Type.—*Rhipicentor bicornis* Nuttall and Warburton, 1908, from North Nyassa, British Central Africa. Host unknown.

Lahille gives the following novel diagram in illustration of his classification:



That there are certain conveniences in this classification may be admitted, but, as Banks (1908, 13) has pointed out, the importance attached to secondary sexual characters hardly carries conviction with it, at least in our present knowledge of ticks. Banks himself has used still another division of the ticks as follows:

TABLE OF THE FAMILIES.

1. No corneous shield on dorsum; head hidden beneath front of body; anus near middle of venter; skin roughened -------- *Argasidæ* 2.
   A corneous shield present on dorsum; head distinct in front of body; anus behind the middle of venter; skin only finely striated -------- *Ixodidæ* 3.

TABLE OF THE GENERA.

2. Margin of body thin and acute -------- *Argas*.
   Margin of body rounded -------- *Ornithodoros*.
3. Venter showing a curved groove a short distance in front of the anus and extending back each side to the hind margin; no posterior marginal festoons; stigmal plate nearly circular; no ocelli; hind coxæ of male not enlarged -------- (*Ixodinæ*) 4.
   Venter showing more or less distinctly a curved groove behind the anus, but none in front of it; the male with distinct marginal festoons, more or less distinct in the female -------- (*Amblyomminæ*) 5.
4. Capitulum slightly angulate on the sides; palpi with the third joint shorter than broad, and broadly rounded -------- *Ceratixodes*.
   Capitulum not angulate on sides; palpi with the third joint longer than broad, and slightly tapering toward the tip -------- *Ixodes*.
5. Sides of capitulum angulate; ocelli present; male with anal plates; palpi very short -------- (*Rhipicephalini*) 6.
   Sides of capitulum not angulate -------- 7.
6. Palpi with acute transverse ridges; stigmal plate nearly circular; porose areas elliptical, distant; no distinct groove behind anus -------- *Margaropus*.
   Palpi without transverse ridges; stigmal plate comma-shaped; porose areas triangular, approximate; a distinct groove behind anus -------- *Rhipicephalus*.
7. Outer angle of the second joint of the short palpi acutely produced; no ocelli; male without anal plates (*Hæmaphysalini*) -------- *Hæmaphysalis*.
   Outer angle of second joint of palpi not acutely produced -------- 8.
8. Palpi longer, second joint about twice as long as broad; coxæ IV of male not enlarged; tarsi II, III, and IV plainly divided, the basal part much shorter than the apical part (*Amblyommini*) -------- *Amblyomma*.
   Palpi shorter, second joint barely longer than broad; coxæ IV of male enlarged; tarsi II, III, and IV indistinctly divided, the parts subequal in length (*Dermacentorini*) -------- *Dermacentor*.

C. W. Howard, government entomologist for Mozambique, has (1908, August) given still another plan of division. He recognizes only one family (*Ixodidæ*) with two subfamilies (*Argasinæ* and *Ixodinæ*). The subfamily *Ixodinæ* he divides as follows:

This subfamily is divided into three tribes, the *Ixodeæ*, *Rhipicephaleæ*, and the *Amblyom[m]eæ*.

Ixodeæ.—Males clothed on all their ventral surface with shields. Anal furrow of both sexes passing around anus in front, and separate from the genital furrows; no eyes. Rostrum elongate. Includes the genus *Ixodes*.

Rhipicephaleæ.—Males provided with one pair of anal shields, ordinarily accompanied by accessory shields. Anal furrow of both sexes passing around the anus behind, and usually joining the genital furrows in front. Eyes present. Rostrum sometimes long and sometimes short. Includes the genera *Rhipicephalus*, *Margaropus*, and *Hyalomma*.

Amblyom[m]eæ.—Males without anal shields. The anal furrow surrounding the anus behind and usually joining the genital furrows in front. Sometimes with eyes. Rostrum long or short. Includes the genera *Amblyomma*, *Aponomma*, *Neuman[n]iella*, *Rhipicentor*, *Dermacentor*, and *Hæmaphysalis*.

The various genera of this subfamily may be separated by the following key:

ADULTS.

A. Males clothed on all their ventral surface with shields; anal furrow of both sexes passing around anus in front, and not joined to the genital furrows ---------------- (*Ixodeæ*), *Ixodes*.

AA. Males with small anal plates or without, but ventral surface *not covered* with plates; anal furrow passing behind anus and usually joining genital furrows, or wanting.

B. Males with two anal plates, usually accompanied by an accessory pair; eyes present ---------------- (*Rhipicephaleæ*.)

C. Rostrum long; palpi elongate and valvate ---------------- *Hyalomma*.

CC. Rostrum short; palpi short, broad, with an outward projection on the second article.

D. Anal groove present, stigmatic plates comma-shaped in both sexes. *Rhipicephalus*.

DD. Anal groove absent; stigmatic plates circular or oval ---- *Margaropus*.

BB. Males with no ventral plates; eyes sometimes present ---- (*Amblyommeæ*.)

C. Eyes present.

D. Palpi long, valvate; coxæ IV not longer in male than coxæ I to III; stigmata triangular ---------------- *Amblyomma*.

DD. Palpi short, thick; coxæ IV much larger in male than coxæ I to III; stigmata comma-shaped, short.

E. Palpi very short and wider than long; coxæ IV with two long spines ---------------- *Rhipicentor*.

EE. Palpi longer than wide; coxæ IV without long spines. *Dermacentor*.

CC. Eyes absent.

D. Palpi long; stigmatic plates comma-shaped.

E. Body as long as wide; anal groove present ---------------- *Aponomma*.

EE. Body wider than long; anal groove not present ---- *Neuman[n]iella*.

DD. Palpi with a sharp projection outward; stigmatic plates circular or short comma-shaped ---------------- *Hæmaphysalis*.

NYMPHS.

(Key based on known Transvaal forms.)

A. Anal groove surrounds the anus in front, opening posteriorly ---------------- *Ixodes*.

AA. Anal groove surrounds the anus behind, opening in front.

B. Palpi produced into a prominent lateral point ---------------- *Hæmaphysalis*.

BB. Palpi more or less cylindrical.

C. Body circular in outline; as wide as long ---------------- *Aponomma*.

CC. Body longer than wide.

D. Body narrower behind than in front ---------------- *Margaropus*.

DD. Body as wide or wider behind than in front.

E. Shield pentagonal in outline; antero-lateral edges occupying three-quarters of the length of the shield ---------------- *Rhipicephalus*.

EE. Shield cordiform in outline.

F. Eyes hemispherical ---------------- *Hyalomma*.

FF. Eyes flat ---------------- *Amblyomma*.

LARVA.

(Key based on known Transvaal forms.)

A. Body much longer than wide; narrowed at both extremities---------*Ixodes*.

AA. Body as long as wide, or longer than wide, but widely rounded behind.

B. Palpi produced into prominent lateral points--------------*Hæmaphysalis*.

BB. Palpi more or less cylindrical.

C. Body as wide as long; circular in outline--------------------*Aponomma*.

CC. Body longer than wide.

D. Palpi very short and thick------------------------------*Margaropus*.

DD. Palpi elongate.

E. Palpi of medium length; more or less pointed at tips; dorsal shield allows a portion of the body to show along its antero-lateral edge, *Rhipicephalus*.

EE. Palpi very long; dorsal shield covers all of anterior portion of body.

F. Eyes hemispherical--------------------------------*Hyalomma*.

FF. Eyes flat---*Amblyomma*.

Whatever general classification may be preferred (and the foregoing summary indicates that the last word on the subject has not yet been written), the genus which interests us at present is *Dermacentor*, which presents the following characters:

Genus DERMACENTOR[a] Koch, 1844.

GENERIC DIAGNOSIS.—*Ixodidæ* (p. 12): Caudal margin bluntly rounded. Color of capitulum and legs usually lighter than body. Dorsum: Scutum emarginate cephalad; subscapular projection present. Eyes present, usually not very prominent. Light-colored rust usually present but variable in different specimens of same species. Punctations usually large and small. Foveolæ present. Venter: Genital pore median, more or less nearly surrounded, especially cephalad, and especially in male, with large and small, prominent punctations with hairs which probably represent sexual sense organs; situated well forward in region of coxæ I or II. Anus median, caudad of plane of coxæ IV; postanal curved groove indistinct; anal ring nearly or quite circular, inclosing two lateral labia with semicircular lateral outline and straight median border, and provided with symmetrically placed hairs. *Stigmal* plates caudo-

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[a] Part of the characters given in this diagnosis will be found to be of more than generic value, but they are inserted here because the entire group is not under discussion.

For other, shorter, diagnoses of this genus, compare the following:

Genus DERMACENTOR Koch.—"Venter showing indistinctly a curved groove behind the anus, from which a median line extends to margin of body. Capitulum not angulate on sides; porose areas elliptic and transverse. Palpi short and broad, the second joint barely longer than broad and with a basal projection above, but not outward. Shield usually marked with white; ocelli present. Coxa I strongly bidentate behind; coxa IV of male much larger than other coxæ, and leg IV larger than other legs. Abdomen shows festoons behind (except in distended female). No anal plates to male. Stigmal plate large, usually reniform in female, more elongate in male. Tarsi II, III, and IV indistinctly lateral of and usually smaller than coxæ IV; round to oval, but usually provided with dorso-lateral prolongations; usually somewhat dissimilar in outline in male and female; consist of three layers; external layer shows goblets, middle layer shows middle portion of goblets and in addition a large number of smaller supporting chitinous cells, which on surface view assume the form of a meshwork; inner layer shows one stem to each goblet. Capitulum: Base rather rectangular, distinctly broader than long, its postero-lateral angles prolonged caudad. Hypostome spatulate, with minute terminal denticles, followed usually by three (in adults) [four in *D. nitens*] longitudinal rows of larger denticles on each half, these followed proximally by scale-like denticles which disappear first from the median, then the middle, then the lateral row. Each palpus longer than broad, but the palpi are only slightly longer than their combined breadth; extend slightly beyond hypostome; lateral margins usually convex; median surface of articles 2 and 3 strongly excavate in long axis; article 1 small, but distinct ventrally; articles 2 and 3 much longer; article 2 broadest; article 4 small, subterminal of article 3 and provided with several bristles; margins, especially ventro-median margin of articles 1 to 3, provided with bristles directed antero-mediad. Legs: Coxæ increase in size I to IV; caudal margin of coxæ I strongly bifid in both sexes, the lateral outer spur narrower and usually longer than the median (inner) blade-like spur; spurs on coxæ II to IV become smaller, especially the median (inner), which may disappear from coxæ IV; trochanter I with dorso-distal retrograde blade-like projection or with prominent retrograde spinous spur; all articles of legs provided with spurs, or bristles or both, paired spurs or knobs on ventral margin may be very prominent but variable in different specimens of same species; tarsi I usually with curved terminal spur, which is better developed on tarsi II to IV; pulvillum extends beyond middle of terminal claws.

Male.—Without anal shields. Much smaller and flatter than gravid female. Sides diverge from scapulæ to maximum breadth usually near coxæ IV or stigmal plate, but divergence may be somewhat interrupted at eyes near legs I; caudad of stigmal plates, convergence usually very rapid. Dorsum: Scutum covers nearly or quite entire dorsum (except capitulum); punctations and hairs present. Cervical grooves more or less distinct; marginal groove usually fairly well marked, forms proximal border of 11 festoons; scutum usually reddish brown, with more or less rust; pseudoscutum usually present. Venter: More or less beset with hairs and pores, which are very noticeably more numerous near (especially anterior of) genital pore (probably sense organ to locate vulva). Genital pore usually between coxæ II; genital groove

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divided, the parts subequal in length, and a minute tooth-like claw at apex. Type: *D. reticulatus* Fabricius.—Banks, 1908, 42.

Dermacentor Koch, 1844.—"Des yeux. Base du rostre plus large que longue, ectangulaire à sa face dorsale. Palpes courts et épais. Péritrèmes en virgule ourte. Face ventrale du mâle dépourvue d'écussons, semblable à celle de la emelle. Hanches de la première paire bidentées dans les deux sexes; celles de a quatrième, chez le mâle, notablement plus grandes que les autres. Ecusson orsal ordinairement orné de dessins variés."—Neumann, 1897a, 360.

Genus Dermacentor Koch, 1844.—"Generic diagnosis.—*Ixodidæ, Rhipiceph-linæ:* Eyes present. Base of capitulum rectangular, broader than long. Dorso-ubmedian porose plates present. Palpi short and thick. Stigmal plate comma-haped; short. Male without anal shields. Coxæ I bidentate in both sexes; oxæ IV of male much larger than I to III. Scutum usually ornamented. Type-species.—*Dermacentor reticulatus* (Fabricius, 1794)."—Salmon and Stiles, 901a, 447.

usually distinct. Stigmal plate not always of same form as in female, but shows same type of goblets. Capitulum: Internal article usually with two visor-like transverse ridges (dorsal process), their free margins directed toward body. Legs: Coxæ I to IV converge toward median line; coxæ IV much larger than others, but may present a large and a small type in one and the same species or even on same specimen; tarsi II to IV with subterminal spur.

Female.—Dorsum: Scutum usually rather prominent because of rust; cervical groove usually of hourglass-like form; eyes at lateral angles. Uncovered portion of dorsum shows punctations (circular pores) with hairs; usually also a distinct median, two submedian, and a more or less distinct marginal groove. Foveolæ caudad of scutum. Venter: Punctations (pores) and fine hairs present; genital grooves diverge more or less gradually to a point about half way between coxæ IV and anus, then more markedly to margins; median, longitudinal, postanal groove usually well marked. Vulva median, in zone of coxæ II or cephalad of this zone. Capitulum: Porose areas present, but may be of somewhat variable outline in a given species.

Type Species.—*D. reticulatus* (Fabricius, 1794) Koch, 1844, on cattle in Europe.

The specimens of *Dermacentor* now in my possession may be divided into four distinct groups according to the microscopic structure of the stigmal plates of the adult.

STRUCTURE OF THE STIGMAL PLATE.[a]

The stigmal plate is a complicated structure. It will be considered here only in so far as it is of importance in differentiating the species or groups of species.

This plate varies to some extent in the two sexes, and it varies also in the different stages of development of one and the same species.

Hexapod larva.—Several authors have mentioned the presence of a stigma caudad of each coxa III. In the hexapod larvæ which I have examined, a similar aperture is present caudad of each coxa I and II. If the aperture caudad of coxæ III is a stigma (and this interpretation is not called into question here), then the others would seem to be stigmata also; accordingly, the hexapod larva of a number of species, at least, is provided with at least three pairs of stigmata. (See also below, p. 21, for a possible fourth pair.)

These stigmata are radically different from the stigmal plates of the adults. In the hexapod of *D. andersoni* (preserved and treated with caustic), for instance, they are small apertures, slightly broader than long, about 6 by 10 μ in superficial diameter, and 12 by 16 μ (outside diameter) in deeper focus; the aperture is provided with a cuticular structure which is slightly differentiated from that of the skin. Both on ventral view and on optical section, these openings are provided with a pair of transverse labial structures, seen upon deeper focus, and apparently of importance in opening and closing the aperture.

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[a] The publication of the present paper has been delayed some months. In the meantime Nuttall, Cooper, and Robinson (1908, December, 347–351) have published an article entitled "On the structure of the spiracles of a tick *Hæmaphysalis punctata* Canestrini and Fanzago," in which they give results for *Hæmaphysalis* similar in many respects to those described here for *Dermacentor*.

A pair of organs, in structure similar to the stigmata, is found ventro-submarginal, laterally in the transverse zone of the anus. It would seem, therefore, that whatever may be the number of stigmata present in other ticks, the hexapod stage of this species (*andersoni*) apparently possesses four pairs.

Adult.—In the adult the stigmal plate is raised slightly above the body cuticle. It varies in size and in outline, the variation, so far as noticed, in the same sex of the same species being much less than the variation, so far as noticed, between the male and female of any one species, or between different species.

In order to arrive at an interpretation of the finer structure of the stigmal plate, so far as is necessary in the present paper, it will be best to take the species formerly considered the American D. *reticulatus*. Banks (1907, 608) has identified this species with *D. albipictus* Packard., with which he also makes *D. variegatus* synonymous. Neumann identifies this form with *D. occidentalis*. It is further interesting to note that there is a specimen of tick labeled "*D. albipictus*" in the United States National Museum which is identical with *D. electus*. The citation of this confusion is not to be interpreted as even an indirect criticism of my colleagues, Neumann and Banks, but as illustrating the difficulties which are encountered in tick determination and as showing how these difficulties may be overcome, at least in part, by the method here described.

I shall recognize the species under consideration as a new species, *Dermacentor salmoni* (see p. 55).

If a transverse section of the stigmal plate (fig. 34) is prepared, or if the plate is mounted so as to show in profile, it will be seen that the plate is composed of three layers, which may be called the external, the middle, and the internal. The differences in these layers are caused by differences in the component parts. Certain large chitinous gobletlike structures are observed. The stem of the goblet is in the internal layer; the rim and the upper portion of the goblet occupy the external layer; the rest of the goblet occupies the middle layer, and is supported by smaller chitinous structures of circular to elongate form. These goblets vary in form in different species, but seem to be fairly constant in each species. Thus in *D. salmoni* the rim of the goblet presents a very broad diameter; in *D. electus* the goblet is very narrow; *D. occidentalis* and *D. andersoni* stand about midway between *D. salmoni* and *D. electus* in respect to this character.

Surface views of the plate naturally vary according to the size of the goblets, and it is this variation which I find to be so useful in differentiating the species in question.

In surface views of *D. salmoni* the three layers described for transverse section can be easily recognized (fig. 32). We notice a superficial layer of very large rings, corresponding to the rim of the goblets; focusing down to the internal layer, we find that for each large ring there is a small circle present, representing a cross section of the stem of the goblet; thus the rings in the external layer of the plate agree in number with the small circles of the internal layer; focusing to the middle layer, it is noticed that there is a somewhat funnel-shaped structure, corresponding to the lower portion of the bowl of the goblet, and the spaces between these funnels are occupied by numerous structures more or less ringlike in section, representing cross sections of the circular to elongate bodies mentioned for the transverse sections of the plate.

In *D. electus*, the rings of the external layer are very small, delicate, very numerous, but often difficult to see (figs. 3, 5); focussing to the internal layer, however, the circles (stems) are seen to be very distinct and very numerous, so that the plate exhibits quite a characteristic appearance; the funnel, so prominent in the middle layer of *D. salmoni*, is not seen on surface view, so that this layer also, in *D. electus*, presents an entirely different appearance.

In *D. andersoni*, the rings (figs. 9, 10) of the external layer stand (in reference to size) between those of *D. salmoni* and *D. electus*; the funnel of the middle layer is present, but not large; the circles of the internal layer are distinct, but less striking than in the case of *D. electus*.

In *D. nitens*, the stigmal plate (figs. 40–43) is very striking. The rings of the external layer are few in number (8 to 20), and relatively large, but they show great variation in size on any one plate and their number may vary on the right and left plates of one individual; the circles of the internal layer are not very distinct; the circles of the middle layer are distinct.

The following table gives the measurements in μ of the goblets observed in a limited number of specimens of the species discussed in this paper.

	Diameters of goblets.				Surface measurements of goblets.			
	Males.		Females.		Males.		Females.	
	Longest.	Shortest.	Longest.	Shortest.	Largest.	Smallest.	Largest.	Smallest.
Reticulatus group:								
D. reticulatus	19	5	16	5	19×14	7×7	17×15	7×6
D. electus	19	5	18	5	19×15	6×5	18×18	6×6
Andersoni group:								
D. occidentalis	40	13	39	15	40×30	17×14	39×30	19×15
D. andersoni	40	5	40	12	37×28	5×5	38×25	14×12
D. venustus	43	12	43	10	43×29	16×15	30×24	12×10
D. parumapertus	....	....	25	7	....	....	18×16	10×9
D. p. marginatus	32	13	30	7	25×20	17×16	30×22	10×9
Salmoni group:								
D. nigrolineatus	53	15	52	17	51×35	25×15	52×40	28×22
D. salmoni	73	26	85	26	70×45	40×30	85×58	39×26
D. variegatus	59	15	49	8	55×36	24×15	49×27	15×8
Nitens group:								
D. nitens	88	43	115	78	85×75	51×51	114×80	89×79

The aperture (macula) of the stigmal plate is of some use in determining the species, but the "chamber" into which this aperture leads is of greater value.

This chamber may be relatively small, as in *D. andersoni* (figs. 9, 10), or it may be very large, as in *D. parumapertus marginatus* (figs. 17–19).

With this general introduction in mind, we may pass to certain species of *Dermacentor*. As work in groups of parasites other than ticks has prevented me from keeping thoroughly informed on the recent extensive literature on the *Ixodoidea*, the following account should not be interpreted as representing a revision of the species of *Dermacentor*, but simply as a contribution to their microscopic anatomy.

Banks (1908, 42–51) recognizes the following North American species of this genus: *D. bifurcatus*, *D. albipictus*, *D. parumapertus*, *D. parumapertus marginatus*, *D. venustus*, *D. occidentalis*, *D. variabilis*, and *D. nitens*. These forms he distinguishes by the following key:

TABLE OF SPECIES.

1. Females -- 2.
   Males -- 8.
2. Stigmal plate nearly circular, with from 10 to 20 very large more or less isolated granulations; shield without distinct punctures; color dark red-brown, without markings ---------------------------- *nitens*.
   Stigmal plate with many much smaller, more crowded granulations; shield distinctly punctured, and usually with some pale markings ------------ 3.
3. Stigmal plate about as broad as long; with short and broad dorsal prolongation, and covered with many very minute granules, scarcely visible as such; shield plainly longer than broad, and much streaked with white - *variabilis*.
   Stigmal plate with much larger granules, at least near the peritreme ---- 4.
4. Stigmal plate without distinct dorsal prolongation; shield plainly longer than broad -- 5.
   Stigmal plate with a more or less distinct dorsal prolongation ----------- 6.

5. Shield mostly white, with brown streaks and spots; porose areas close together ---- *albipictus.*
   Shield dark red-brown, with very little white; porose areas rather widely separated ---- *nigrolineatus.*
6. **Shield mostly white, about as broad as long** ---- **7.**
   Shield without white, or but little, plainly a little longer than broad; porose areas but little longer than broad, and well separated ---- *parumapertus.*
7. Porose areas very small; stigmal plate with rather wide dorsal prolongation ---- *occidentalis.*
   Porose areas larger; stigmal plate with a more narrow dorsal prolongation ---- *venustus.*
8. Stigmal plate with from 4 to 10 very large isolated granules; dorsum without white marks; only eight impressed lines behind ---- ***nitens.***
   Stigmal plate with many smaller, more crowded granules; twelve impressed lines behind ---- **9.**
9. Stigmal plate about as broad behind as long, with dorsal prolongation, the granulations extremely minute; dorsum marked with white streaks and spots ---- *variabilis.*
   Stigmal plate usually plainly longer than broad; the granulations much larger ---- **10.**
10. Stigmal plate without distinct apical prolongation; the sides of the body more nearly parallel ---- **11.**
    Stigmal plate with distinct apical prolongation; sides of body more divergent ---- **12.**
11. Dorsum mostly white, with brown streaks and spots in a pattern; hind angles of the capitulum but little produced; coxa IV about one-half as long as broad on base; large species ---- ***albipictus.***
    Dorsum red-brown, with black lines, no white; hind angles of capitulum much prolonged; coxa IV not twice as broad on base as long; species of moderate size ---- ***nigrolineatus.***
12. Dorsum with few, if any, white spots; coxa IV about as long as broad at base; hind angles of capitulum moderately produced ---- *parumapertus.*
    Dorsum largely white, or much spotted with white ---- **13.**
13. Stigmal plate more attenuate behind; coxa IV about one-half as long as broad at base; hind angles of capitulum moderately produced ---- *venustus.*
    Stigmal plate less attenuate behind; coxa IV not twice as broad on base as long; hind angles of capitulum much produced ---- *occidentalis.*

It will be noticed that Banks has utilized the "granulations" (goblets) of the stigmal plates in a manner somewhat similar to the way Salmon and Stiles (1901a) used them. He has also placed considerable stress on the size of coxæ IV. Further, the rust and the prolongations of the stigmal plate are used in his keys.

In the present paper, greater stress will be placed upon the goblets (granulations); the rust and the stigmal prolongations may be utilized, but with reserve; the size of coxæ IV will not be given much prominence, as in my experience this is subject to extreme variation and has too frequently misled me.

D. bifurcatus from a wild cat, in Texas, is not at my disposal.

D. albipictus as interpreted by Banks represents at least two species, namely, *D. variegatus* [=*albipictus*] and *D. salmoni.*

D. parumapertus is undoubtedly a good species, but my material is not sufficient to permit as careful a study as I should wish.

D. parumapertus marginatus is present in my material.

D. venustus as interpreted by Banks contains two species, *D. venustus* of Texas and *D. andersoni* of Montana.

D. occidentalis is a good species.

D. nigrolineatus is closely allied to *D. salmoni*, but is a distinct species.

D. variabilis seu *electus* presents certain variations which may possibly prove to be of subspecific or possibly even of specific value.

D. nitens is so distinct from the other species that the probability is present that it will eventually be taken as basis for a distinct subgenus.

The following key may be utilized to distinguish the species discussed in the present paper:

KEY TO CERTAIN SPECIES OF DERMACENTOR.

1. Adults with four longitudinal rows of large denticles on each half of hypostome; stigmal plate nearly circular, without dorso-lateral prolongation, goblets very large, attaining 43 to 115 μ in diameter; not over 40 per plate, each surrounded by an elevated row of regularly arranged supporting cells; white rust wanting; base of capitulum distinctly broader than long, its postero-lateral angles prolonged slightly, if at all; coxæ I with short spurs; trochanter I with small dorso-terminal blade; (Nitens group) -- *D. nitens, p.* 63.

   Male: Stigmal plate slightly longer than broad, with 4 to 11 goblets; genital pore surrounded by numerous punctations and hairs, a double transverse row immediately caudad of pore.

   Female: Stigmal plate nearly circular, with 6 to 20 goblets; genital pore (? apparently with fewer hairs than in male).

   Adults with three longitudinal rows of large denticles on each half of hypostome; goblets small, medium, or large, always more than 40 per plate; whitish rust usually present -- 2.

2. Dorso-lateral prolongation of stigmal plate small or absent; plates of adults distinctly longer than broad; goblets large, usually 30 to 85 μ in diameter, appearing as very coarse punctations on untreated specimens, but on specimens treated with caustic they appear very distinct in outline; base of capitulum distinctly (usually about twice) broader than long, the postero-lateral angles distinctly prolonged caudad; spurs of coxæ I long, lateral spur slightly longer than median; trochanter I with dorso-terminal spur -- *Salmoni* group, 8.

   Dorso-lateral prolongation of stigmal plate distinct; body of plate distinctly longer than broad; goblets of medium size, usually 17.5 to 35 or 40 μ in diameter, appearing as medium sized punctations on untreated specimens, but on specimens treated with caustic they appear very distinct in outline, which is not usually circular; base of capitulum usually less than twice as broad as long, the postero-lateral angles always distinctly prolonged caudad -- *Andersoni* group, 4.

Dorso-lateral prolongation of stigmal plate distinct; goblets small, rarely exceeding 17.6 μ, occasionally reaching 19 μ in diameter; on untreated specimens they appear as very fine granulations, and on specimens treated with caustic they may be difficult to see, but their large number can be determined from the prominent stems of the goblets; surface of outline of goblets distinctly circular; base of capitulum usually less than twice as broad as long, the postero-lateral angles distinctly prolonged caudad; spurs of coxæ I long---------------------*Reticulatus* group, 3.

RETICULATUS GROUP.

3. Article 2 of palpi with dorso-lateral retrograde spur, sometimes very prominent; palpi with tendency to angular sides; trochanter I with tendency to distinct dorso-terminal retrograde spur; stigmal plate with tendency to distinct elongation, especially in male, with more or less distinctly terminal prolongation, and with rather broad margin free of goblets; type locality Europe------------------------------*D. reticulatus*, p. 28.

Male: Plate long and narrow, goblets about 375 per plate, 5 to 19 μ in diameter; genital pore surrounded by numerous large and small punctations.

Female: Plate not so long as in male, relatively broader; goblets about 200 per plate, 5 to 16 μ in diameter; genital pore surrounded by large and numerous smaller punctations.

Article 2 of palpi with generally not very prominent dorso-median retrograde prolongation; palpi with tendency to convex sides; trochanter I with rather indistinct spur, often almost resembling a blade; stigmal plate with decided tendency to broadness, especially in female, with tendency to subterminal prolongation, and with much narrower or no margin free of goblets; goblets may attain 535 in number and 5 to 19 μ in diameter; type locality, United States---------------------------*D. electus*, p. 29.

Male: Plate much shorter than in *D. reticulatus*, and with smaller aperture; aperture smaller than in female *electus*; about 535 goblets per plate, 5 to 19 μ in diameter; genital pore with large and small punctations.

Female: Plate usually extremely broad, with large aperture, and decidedly subterminal prolongation; about 450 goblets per plate, 5 to 18 μ in diameter; genital pore with large and smaller punctations.

ANDERSONI GROUP.

4. Trochanter I with distinct dorso-subterminal retrograde, sharp, digitate spur; postero-lateral angles of capitulum pronouncedly prolonged caudad, 112 to 160 μ long; goblets attain 13 to 40 μ in diameter; type locality, California---------------------------------*D. occidentalis*, p. 32.

Male: Stigmal plate elongate, small, with about 65 goblets, which attain 13 to 40 μ in diameter.

Female: Stigmal plate much broader, with about 70 goblets, which attain 15 to 39 μ in diameter.

Trochanter I with dorso-subterminal blade; postero-lateral angles of capitulum with rather short prolongations:

Male--5.

Female--6.

5. Stigmal plate small, prolongation forming very obtuse angle with plate; goblets about 45 per plate, scattered, not covering entire plate, not angular, but nearly circular; goblets attain 44 in number, 13 to 32 μ in diameter; coxæ I with unusually short spurs, the inner considerably shorter than outer; scutum with very little rust; small species, type locality, Arizona ---------- *D. parumapertus marginatus*, p. 48.

   Stigmal plate twice as large; broad prolongation, forming rounded, somewhat obtuse angle with plate; goblets about 75 per plate, scattered, those near aperture somewhat elongate, those in prolongation more circular, may attain 12 to 43 μ in diameter; coxæ I with longer spurs, inner slightly shorter than outer; scutum with considerable rust; type locality, Texas ---------- *D. venustus*, p. 43.

   Stigmal plate still larger; prolongation shows decided tendency to form right angle; goblets, about 157 per plate, attain 5 to 32 or 40 μ in diameter, tendency to angularity; coxæ I with long spurs, inner slightly shorter than outer; scutum with considerable rust; type locality, Bitter Root Valley, Arizona ---------- *D. andersoni*, p. 36.

6. Stigmal plate small, its chamber relatively unusually large, occupying nearly entire body of plate; a portion of dorsal margin without goblets or supporting cells; goblets around aperture with unusual tendency to circular outline; about 90 goblets per plate; prolongation with few goblets; scutum with little rust; coxæ I with short spurs, the inner distinctly shorter than the outer:

   D. parumapertus: Type form from Lakeside, Cal., requires further study; apparently dorsal margin of plate narrow, about 90 goblets of 7 to 25 μ diameter, prolongation shows tendency to form very acute angle, little rust in scutum ---------- p. 46.

   D. parumapertus marginatus: Broad triangular dorsal margin of plate without goblets; goblets 73 to 99 in number, 7 to 30 μ in diameter, prolongation shows tendency to form acute angle; very little rust on scutum, chiefly present near margin; type locality Arizona ---------- p. 48.

   Stigmal plate larger, chamber relatively smaller, does not occupy nearly all of body of plate; very little if any of dorsal margin free of supporting cells; goblets around aperture show decided tendency to angularity; scutum with considerable rust; coxæ I with long spines, inner slightly shorter than outer ---------- 7.

7. About 105 goblets per plate, attain 10 to 43 μ in diameter, apparently absent from marginal band; requires further study; type locality Texas.
   D. venustus, p. 43.

   About 120 goblets per plate, attain 12 to 32 or 40 μ in diameter, absent from narrow marginal band; prolongation shows decided tendency to form an acute angle with plate; type locality Bitter Root Valley, Montana.
   D. andersoni, p. 36.

SALMONI GROUP.

8. Goblets may exceed 100 in number, are crowded together, and attain 15 to 59 μ in diameter; aperture equatorial; body elongate, quite pilose, rust present; capitulum and coxæ I large; type locality North America.
   D. variegatus, p. 60.

   Male: Pseudoscutum distinct; goblets attain 126 in number, 15 to 59 μ in diameter.

   Female: Scutum large, 1.7 to 2.1 mm. long; goblets attain 108 in number, 8 to 49 μ in diameter.

   Goblets do not exceed 90 in number ---------- 9.

9. Goblets 51 to 79 in number, and 26 to 85 μ in diameter; aperture usually equatorial; body not so elongate and pilose as in *variegatus*, rust present; capitulum and coxæ I smaller than in *variegatus;* stigmal plate rather irregular in outline; type locality Oklahoma.........*D. salmoni*, p. 55.
   Male: Pseudoscutum not distinct; goblets 51 to 56 in number, 26 to 73 μ in diameter.
   Female: Scutum large, 1.3 to 2 mm. long; goblets attain 71 to 79 in number, 26 to 85 μ in diameter.
   Goblets about 70 in number, 15 to 52 μ in diameter; small species, quite pilose but without rust; capitulum and coxæ I small; type locality Adirondacks, N. Y.................................*D. nigrolineatus*, p. 51.
   Male: Pseudoscutum very indistinct or absent; stigmal plate elongate with preequatorial aperture; goblets about 69 in number, 15 to 53 μ in diameter.
   Female: Scutum small, 1.1 to 1.4 mm. long; stigmal plate irregularly circular; goblets 66 to 103 in number, 17 to 52 μ in diameter.

RETICULATUS GROUP.

Dermacentor reticulatus and *D. electus* [=*D. variabilis* (Say) Banks] show small goblet cells of very nearly the same size, rarely exceeding, in the cases I have thus far measured, 17.6 μ in diameter. On account of their small diameter they are not prominent, and hence they can be easily overlooked, but if a lower focus is taken the stems of the goblets (in the inner layer) come prominently into view.

DERMACENTOR RETICULATUS (Fabricius, 1794) Koch, 1844.

(Figs. 1, 2, 49, 50, 71, 82, 83, 101, 102, 120, 121.)

1794: *Acarus reticulatus* Fabricius, 1794a, 428 (host ? ; Europe).

1844: *Dermacentor reticulatus* (Fabricius, 1794) Koch, 1844a, 235.—Neumann, 1901a, 340 (names *Bos taurus* as type host: France).—Salmon and Stiles, 1901a, 448–452 *in part only* (namely, exclusive of American specimens and of figs. 61, 169–177).

The four specimens here taken as *D. reticulatus* are accepted as such on basis of determination by Neumann. They bear the number U. S. B. A. I. 3904, were collected in France, and represent two males and two females.

One male has a very prominent dorsal retrograde spinous prolongation on the second palpal article (fig. 49), but this is very much less evident on one female, and is not evident on the other male and female. The palpi of the male with the retrograde prolongation are very pronouncedly swollen laterally and one female agrees in this character; the other male and the other female have palpi which are much narrower. Either these palpal characters would therefore appear to be of less value than has heretofore been assumed, or ultimate investigations may show that two species are still confused in the European *D. reticulatus;* the pair without the retrograde spine is strongly suggestive of *D. electus*.

The stigmal plates are shown in figure 1 (male) and figure 2 (female).

It will be noticed that these plates are not identical in outline. That of the male is much more elongate than that of the female. They agree, however, in certain important characters, namely: The goblets present approximately the same relative distribution, being closer together near the aperture, but somewhat more separated and decidedly smaller near the periphery. Numerous (215 to 378) goblets are present, and these attain a maximum diameter of 19 μ, but the peripheral goblets may be as small as 7 μ.

The longest diameter of the male plate is 848 μ; the greatest breadth 480 μ. The length of the female plate is 656 μ; its breadth is 464 μ.

If it should eventually develop that the present *D. reticulatus* of Europe contains two species, the name should be confined to forms with stigmal plates as here described and with a prominent dorsal retrograde spinous prolongation on the second palpal article.

Neumann has designated *Bos taurus* as type host and France as type locality for this species.

DERMACENTOR VARIABILIS (Say, 1821) Bank, 1907=DERMACENTOR ELECTUS Koch, 1844.

(Figs. 3-6, 51, 52, 72, 84, 85, 103, 104, 122, 123.)

1821: *Ixodes variabilis* Say, 1821, 77.
1844: *Dermacentor electus* Koch, 1844a, 235 (type locality, Pennsylvania); 1847a, 109, pl. 22, figs. 83-84.
1907: *Dermacentor variabilis* (Say, 1821) Banks, 1907, 608; 1908, 49-50, pl. 7, figs. 3-4, 6, 8, pl. 10, figs. 3-4.

As basis of this species I have taken a series of specimens determined as *D. electus* by Neumann. Some of these were determined independently as *D. electus* by myself and agree with the form which Salmon and Stiles (1901a, 455-456, figs. 186-214) published under this name.

Banks (1907, 608; 1908, 49-50) makes this species identical with Say's *Ixodes variabilis* 1821, and he adopts the name *D. variabilis*. Banks informs me that this identification is based upon comparison of descriptions, not upon comparison of types. Personally I have formed no opinion as yet upon the correctness of this synonymy. The form which I here discuss is *D. electus;* if Banks is correct in his view, Say's specific name takes priority.

The stigmal plates of *D. electus* [=*D. variabilis* (Say) Banks] are very similar, in respect to the size of the goblets, to those of *D. reticulatus*, although the distribution of the goblets is somewhat different, and the outline of the plates also presents marked differences.

The male plate is not so pronouncedly elongate as the male plate of *D. reticulatus* and in the female plate the "comma" is more equatorial while in the female *D. reticulatus* it is nearly terminal.

It will be noticed in figures 3 and 4, of the male plate, that the tail of the "comma" is nearly terminal.

Comparing the plates of *D. electus* with those of *D. reticulatus*, it will be noticed that the goblets are more numerous, and more generally distributed in the former and extend quite to the periphery of the plate, while in *D. reticulatus* they do not attain the periphery. In some specimens of *D. electus*, considerable variation was found in respect to this point.

In *D. electus*, the goblets are 455 to 536 in number, may attain 16 μ or even 19 μ in diameter. It is, however, often quite difficult to see the upper portion of the goblets (figs. 3 and 5) although it is very easy to see the lower stems (fig. 4) which naturally give a clear idea of the distribution; these stems may attain 5 to 6 μ or even 8 μ in diameter.

The chamber under the breathing pore is relatively small, elongate, and near the ventro-median margin of the plate. The plates may attain 608 to 672 μ long by 420 μ broad in the male, and 512 to 752 μ long by 480 to 640 μ broad in the female.

In connection with the foregoing observations the following specimens have been examined:

U. S. B. A. I. 2131, from skin. Examined by Neumann in connection with his revision of ticks and recognized as *D. electus*.

U. S. B. A. I. 2156, collected by Cooper Curtice and determined by him as *D. americanus*; reexamined by Chapman and determined as *D. electus*.

U. S. B. A. I. 2157, collected by Dr. B. G. Wilder, from *Canis familiaris* at Siasconset, Nantucket, Mass., July, 1889; determined by Neumann as *D. americanus* [= *electus*].

U. S. B. A. I. 2158, collected by Hassall, July 12, 1892, from *Canis familiaris*; determined by Neumann, February 4, 1897, as *D. americanus* [= *electus*].

U. S. B. A. I. 3011, collected in 1898 from *Bos taurus*, at St. Joseph, Mo.; determined 1901 by Stiles as *D. electus*.

U. S. B. A. I. 3150, collected May 25, 1899, by Mrs. R. E. Miller, from *Canis familiaris*, at Joppa, Md.; determined 1899 by Stiles and Hassall as *D. americanus* [= *electus*].

U. S. B. A. I. 3205. Stigmal plate of unusual outline.

U. S. B. A. I. 3233, collected in 1901 by W. S. D. Haines, from *Canis familiaris*, at Joppa, Md.; determined 1901 by Stiles as *D. electus*.

U. S. B. A. I. 3403, collected May, 1902, by Ephriam Cutter, from *Canis familiaris*, at West Falmouth, Buzzards Bay, Mass.; determined 1902 by Stiles as *D. electus*.

U. S. B. A. I. 3518, collected July 3, 1903, by Dr. J. R. Mohler, from *Canis familiaris*, at Washington, D. C.; determined July 3, 1903, by Ransom as D. *electus*.

U. S. B. A. I. 3529, collected August, 1903, by J. S. Jackson, from *Bos taurus*, at San Luis Obispo, Cal.; determined by Ransom as *D. electus*.

U. S. B. A. I. 4037 (from 2126), collected October, 1890, by Cooper Curtice, from *Bos taurus*, at Albany, Tex.; determined by Neumann as *D. electus*.

U. S. B. A. I. 4138, collected from *Bos taurus* in Maryland; determined June 15, 1905, as *D. electus*.

U. S. B. A. I. 4360, collected from wild cat, California.

The following specimens bear numbers of the U. S. P. H. & M. H. S.:

U. S. P. H. & M. H. S. 9746, female belonging to U. S. Nat. Mus. (old number 2381), from (? S. C.) Birdslee, Gainsville, Ohio, July 5, 1883; determined January 4, 1906, by Stiles as *D. electus*.

U. S. P. H. & M. H. S. 9758, female belonging to U. S. Nat. Mus. (old number 5760); determined by Stiles as *D. electus*.

U. S. P. H. & M. H. S. 9773, male belonging to U. S. Nat. Mus. (no number); collected in Virginia, June 12, 1881.

U. S. P. H. & M. H. S. 9781, male belonging to U. S. Nat. Mus.; original label reads "506, swept from grass, Coney Island, L. I., J. L. Zabriskie, 27. V. 1891; *Ixodes albipictus* Pack.;" the determination was apparently made by Ashmead; redetermined *D. electus* by Stiles.

U. S. P. H. & M. H. S. 9920, collected August 17, 1905, by S. Goes, from *Canis familiaris*, at Gurley, Tex.; determined by Stiles.

U. S. P. H. & M. H. S. 10624, locality and host unknown.

The following specimens are from Marx Collection (now in the U. S. Nat. Mus.); they were determined by Marx, and later redetermined by Neumann as *D. electus* or *D. americanus:*

Marx 123, from Mexico.

Marx 124, host *Canis familiaris;* Nantucket.

Marx 125, host panther; Florida.

Marx 126, host *Canis familiaris;* D. C.

Marx 127, host *Canis familiaris;* D. C.

Marx 128, host *Canis familiaris;* one specimen, from Labrador. So far as can be judged from unmounted material, the determination seems to be correct.

Marx 130. Minnesota. Same as preceding number.

Marx 131. Alabama. Ditto.

Marx 132. Alognakig, Alaska. Ditto.

Marx 133. Host *Canis familiaris;* Nantucket.

Marx 134. Host *Equus caballus;* Kansas.

Marx 136. San Jacinto, Cal.

Marx 140. Host and locality not given. Examined by Neumann, but apparently not by Marx.

Marx 141. Ditto.

As already indicated, the foregoing specimens present considerable variation in certain characters, and it is by no means excluded that the American *D. electus* will eventually be subdivided into several distinct species or subspecies. Our material does not contain a sufficient number of specimens from any one given locality to enable us to conclude safely whether the variations noticed are individual or specific.

ANDERSONI GROUP.

We now pass to a group in which the goblets are of medium size, usually varying from about 17.6 to 35 μ or 40 μ in diameter.

DERMACENTOR OCCIDENTALIS Marx, 1892.

(Figs. 7, 8, 53, 54, 73, 86, 87, 105, 106, 124, 125.)

1892: *Dermacentor occidentalis* Marx in Curtice, 1892g, 226 (on *Bos taurus;* California), 234 (on cattle, deer, dog, and man); 1892i, 237.—Banks, 1907, 608; 1908, 47-48, pl. 8, figs. 1-2.—Morgan, 1899a, 134.—Neumann, 1897a, 365 (as syn. of *D. reticulatus*); 1905d, 235 (var. of *D. reticulatus*).—Railliet, 1893a, 714.—Salmon and Stiles, 1901a, 449 (syn. of *D. reticulatus*).

1905: *D. reticulatus occidentalis* (Marx, 1892) Neumann, 1895d, 235-236.

Not: *D. occidentalis* of Montana, mentioned in connection with Rocky Mountain Spotted Fever.

Specific diagnosis.—*Dermacentor* (p. 18): ♂ and young ♀: Caudal margin nearly semicircular. Color (alcohol specimens) light to dark reddish-brown, except for rust on scutum; capitulum and legs lighter. Dorsum: Eyes not prominent. Scutum with whitish rust (alcohol specimens) punctations large and small. Venter: Genital pore surrounded by numerous hairs (♂) which are much less prominent and less numerous in female than in female of *D. andersoni.* Anal ring circular or slightly broader than long, diameters vary between 192 and 272 μ. Stigmal plates with prominent dorso-lateral prolongation, which shows a tendency to form at the caudal margin of the plate a broadly rounded obtuse angle in the male and an acute angle in the female; aperture elongate, relatively not quite so long as in *D. andersoni;* goblets of medium size, attain about 32 μ, exceptionally 40 μ, in diameter, are closely set, but may be not so closely set as in *D. andersoni*, and occupy nearly entire surface except margin and distal portion of prolongation; meshes of middle layer about 9 μ in diameter; stem of goblets about 5 to 6 μ in diameter. Capitulum: Postero-lateral projections of base long and prominent, giving to posterior margin (especially of male) a prominently concave line. External article, see male. Hypostome, each half with a number of minute subterminal denticles, followed by three rows of larger denticles, followed proximally by smaller scale-like denticles. Palpi, lateral margins somewhat convex, but for part of the distance nearly straight; four to five strong bristles on ventro-median margin of article 1; article 3 not distinctly triangular dorsally, may even appear rather quadrangular; few bristles on concave margin of dorso-terminal portion, terminal bristles present; an indication of a dorso-retrograde prolongation at postero-median angle of article 2. Legs: Lateral (outer) spur of coxae I longer than the median (inner) spur; trochanter I with strong and very prominent sharp retrograde spur; spurs or knobs on ventral margins of femur IV, tibia III-IV, and protarsus IV, variable in different specimens, see male; tarsi I with very small terminal spur.

Male.—Length, 3.68 to 4.5 mm. (exclusive of hind legs); greatest breadth, 2.0 to 2.56 mm. Body, oval; unmounted specimens attain 0.8 to 0.86 mm. broad at scapulae, specimens mounted under pressure may measure up to 0.88 mm. at this point; the sides diverge from here until they reach their maximum diameter at coxae IV to anterior margin of stigmal plate, where they vary from 2.0 to 2.56 mm. in unmounted specimens. Dorsum: Scutum covers nearly or quite entire dorsum (except capitulum); scapulae project 0.20 to 0.32 mm. from border of excavation; cervical grooves much more distinct anteriorly than posteriorly; marginal groove rather indistinct; scutum reddish brown, nearly covered with thin rust; pseudoscutum distinct but not very prominent, extending about 0.40 of length of scutum in median line; red spots not so prominent (alcohol specimens) as in *D. andersoni*, and rather variable, the most prominent are: (a) Four short spots arranged radially or nearly so, from caudal margin of pseudoscutum; (b) two spots back of these; (c) a narrow elongate median and two shorter somewhat elongate submedian spots; (d) the forked spot somewhat indistinct, may also be nil; dorso-marginal spots very variable, several may be seen each side or they may be very indistinct; the rust on the eleven distinct postero-marginal festoons exceedingly variable; numerous small and a few larger punctations present; minute hairs issue from many of these punctations. Foveolæ a short distance caudal of pseudoscutum, dorsal of or near median margin of coxæ IV, and measuring 48 to 64 μ or even 80 μ in diameter; in one case, one foveola was divided into two. Venter: Ventral surface sparsely beset with hairs which, with the circular pores through which they pass, are noticeably more numerous around the genital pore. Genital pore between coxæ II; genital groove distinct. Stigmal plate 0.320 to 0.512 mm. long (through the aperture); 0.256 to 0.440 mm. broad (including prolongation); 0.40 to 0.56 mm. from antero-median margin to tip of prolongation; goblets may attain about 65 in number and 13 to 40 μ in diameter. Capitulum: 0.46 to 0.688 mm. long; base 0.40 to 0.528 mm. broad, and 0.224 to 0.30 mm. from posterior margin to anterior margin of portion over palpi; postero-lateral prolongations 112 to 160 μ in length, rather sharp. Mandibles 0.768 to 0.832 mm. long; digit 112 to 128 μ long; external article with proximally, rather large recurved tooth, a small subapical tooth, and an exceedingly small, almost invisible apical tooth; dorsal process of internal article elongate transversely with two visor-like transverse ridges, similar to *D. andersoni.* Hypostome with seven to eight large denticles in each row. Palpi 0.40 to 0.432 mm. long, each palpus 208 to 272 μ in maximum breadth; article 2 with four to five bristles on ventro-median edge; article 3 with one to two bristles on ventro-median edge. Legs: Coxæ IV present two types; they may be relatively short or long; tibia III, and femur, tibia, and protarsus IV provided with a double row of three prominent ventral knobs or spurs, especially large on tibia IV; femur IV also with a small additional ventral pair of spurs; tarsi I with terminal recurved spur, variable, may be practically nil, or may be rather well developed; tarsi II to IV with subterminal spur, variable in different specimens.

Young female.—Length 4.0 to 4.25 (exclusive of hind legs: with hind legs extended may attain 5.5 mm.); greatest breadth 2.37 mm. Body elongate oval; unmounted specimens 0.91 to 0.99 mm. broad at scapulæ, specimens mounted under pressure may measure up to (?) at this point; the sides diverge from here to a maximum breadth about at coxæ IV to stigmal plate. Dorsum: Scutum covers 0.40 to 0.46 of length of body (exclusive of head); 1.31 to 1.56 mm. long in median line; 1.5 to 1.7 mm. broad at lateral angles; quite prominent because of its whitish rust; scapulæ project about 0.22 to 0.28 mm. from border of excavation, which is somewhat convex cephalad; from shoulders, the sides of scutum diverge in distinctly curved convex lines to lateral angles, then converge rapidly in nearly straight lines to the rounded postero-lateral angles, when they converge more rapidly, and form a bluntly rounded posterior angle. Cervical grooves decidedly deeper anteriorly than posteriorly, each forming mediad the concave side of an hourglass, but inclining to a curved rather than a straight line; because of the depth of these grooves anteriorly the antero-median field is very prominent and rather raised; the distal diverging portion may be paralleled laterally by an indistinct dark line; rust not so thick in antero-median field as elsewhere, and absent from a portion of the margin caudad of lateral angles. Eyes convex, distinct, but not prominent, at lateral angles; translucent in cleared specimens. Uncovered portion of dorsum shows numerous finer punctuations (circular pores) with minute hairs and fewer coarser punctations (circular pores); also a distinct median, two submedian grooves, and distinct marginal groove limiting the 11 postero-marginal festoons. Foveolæ 72μ in diameter, a short distance caudad of scutum. Venter: Punctations and fine hairs present; genital grooves diverge gradually but regularly to a point about half way between coxæ IV and anus, then more markedly to margins; median postanal groove distinct. Stigmal plate 0.36 to 0.384 mm. long (measured through aperture; one specimen); breadth (including prolongation) about the same; goblets may attain about 70 in number, and 15 to 39μ in diameter. Vulva between coxæ II. Capitulum: 0.656 to 0.72 mm. long; base 0.576 to 0.656 mm. broad, and 0.23 to 0.27 mm. from posterior margin to anterior margin of portion over palpi; postero-lateral prolongations 112μ long (one specimen), bluntly pointed; porose areas do not meet in median line. Mandibles ?; external article apparently as in male; dorsal process of internal article apparently as in *D. andersoni.* Smaller anterior denticles of hypostome confined to smaller area than in male; eight to ten large denticles in each row; proximal denticles not so small as in male. Palpi 0.528 to 0.560 mm. long; 208 to 272 mm. broad; article 2 with six, article 3 with two to three bristles on ventro-median edge. Legs: Coxæ I to IV diverge slightly from median line; knobs and spurs on tibia III, and on femur, tibia, and protarsus IV not so well developed as in male; tarsi I with very small terminal spur which may be almost nil; tarsi II to IV with terminal spur, also with small ventral knobs.

Replete female.—One specimen, partially replete. Length, 6.5 mm.; maximum breadth, 4 mm.; legs I lateral of scutum. Venter: Vulva has shifted slightly forward of coxæ II. Legs: Coxæ farther apart than in young female, due to repletion.

Type.—Marx 119, U. S. Nat. Mus.

Hosts.—Deer, cattle, dogs, and man; California.

Curtice (1892g, 226, 234) gives, in regard to this tick, the following statements:

> P. 226: "*Dermacentor occidentalis* Marx in MSS., the cattle tick of California. This cattle tick is like *D. americanus* [= *electus*], but is somewhat smaller; the head shield of the female is decidedly whiter, the shield of the male being nearly white with small symmetrically situated dark spots toward either side."
>
> P. 234: "*Dermacentor occidentalis* Marx, MS. This California species, the one ordinarily found on deer, cattle, and dogs, was quite annoying to me during the past season. They get on one while passing through the chaparal and arriving at the base of the hair insidiously insert their beak into the skin."

Later, Curtice (1892i, 237) merely mentions "*D. occidentalis* Marx, the western deer tick," as having been taken on cattle.

Morgan (1899, 134) refers to "the deer tick (perhaps *Dermacentor occidentalis*)" as being recognized as distinct by hunters in Louisiana. A legitimate doubt may arise as to whether the Californian species occurs in Louisiana.

Neumann (1897a, 365), on basis of an examination of males and females, collected "sur le Daim" in California, and labeled by Marx as *D. occidentalis*, considered this form identical with *D. reticulatus* of Europe. Later, Neumann (1905d, 235-236) reexamined these specimens and recognized them as a subspecies, *D. reticulatus occidentalis.* Through error he identified with this form the American

D. reticulatus of Salmon and Stiles. He gives the following characters for Marx's specimens:

> Base of rostrum a little broader, with posterior angles very much prolonged backward. Hypostome with six rows of denticles. Various prominences of the palpi slightly pronounced. Granulations (here=goblets) of the peritremes (=stigmal plates) much more apparent. Coxæ II–IV with longer spine.
>
> *Male.*—Coxæ IV sometimes prolonged caudad to the anus.
>
> *Female.*—Dorsal shield relatively longer. Porose areas smaller.
>
> California and Tennessee. On *Cariacus canadensis* (Briss.), *Bos taurus* L., and *Equus caballus* L.

Banks (1907, 608) lists *D. occidentalis* as a distinct species, but gives no anatomical details. Later, however (1908, 47–48), he gives the following specific description:

> *Male.*—Red-brown, with many waxy-white markings, often with a waxy bloom, sometimes almost wholly white, but there is a red-brown near the eyes, on the festoons, and several submedian spots; moreover, the white is broken by the many red-brown punctures; legs pale reddish brown, marked with white above. Capitulum rather narrow, and the hind angles prolonged into very prominent spines; palpi very short, not as long as the width of the capitulum; dorsum not much more than one and one-half times as long as broad, with many punctures, but mostly small; lateral furrows distinct and long, twelve indented lines behind. Legs of moderate size, tarsus IV with two very distinct teeth below and one less prominent, teeth on other joints distinct; coxæ armed as usual; coxæ IV about one and one-half as wide at base as long. Stigmal plate elongate, with a broad, turned-up tip, almost truncate; large granulations on the main part, small ones on the tip.
>
> Length of male, 3 to 3.5 mm.
>
> *Female.*—Shield red-brown, mostly covered with white, red-brown near eyes and in the middle region, and the white broken up by the many brown dots at punctures; capitulum and legs red-brown, latter white at tips of joints, and generally paler above than below; abdomen dark red-brown. Capitulum rather small, and hind angles prominent, and the porose areas very small and rather close together. Shield about as broad as long, broadest before middle, and rather pointed behind, with many small punctures and some larger, but not nearly as many large ones as in *D. parumapertus.* Legs rather small, coxæ armed as usual. Stigmal plate with a broad dorsal prolongation, with large granulations in the main part, and minute ones on the prolongation.
>
> Length of female shield, 1.5 mm.
>
> Nearly all specimens come from California—Occidental, San Diego, Goose Lake, Siskiyou County, Santa Clara County, Humboldt County; some taken from deer.
>
> Closely related to *D. venustus*, but with a more narrow capitulum, and with a broader prolongation to stigmal plate, in the male by shorter hind coxæ and in the female by smaller porose areas. Taken together, I think these characters indicate its distinctness from *D. venustus.*

In Marx's material I find one bottle (Marx 119) of specimens labeled *D. occidentalis*, from Occidental, California; this I assume is the material upon which he based the name, hence this would be the type material.

A microscopic examination of the stigmal plate shows that this tick is different not only from *D. reticulatus* of Europe but also from the American *D. reticulatus* of Salmon and Stiles (=*D. salmoni*).

Fig. 7 shows the male plate, with a pronounced dorso-lateral prolongation. The length of the plate, through the aperture, is 384 μ; the breadth, including the prolongation, 320 μ. The goblets are 67 in number, of medium size, attaining about 32 μ in diameter, rather closely packed together so that they have a tendency to lose their circular form for an angular outline. The aperture of the plate is relatively small when compared with *D. andersoni*.

The following material, which has been taken as basis for the specific diagnosis given in the foregoing, has been examined:

Marx 115. Host ?; Kern County, Cal., determined by Marx as *D. occidentalis*; determined by Neumann as *D. reticulatus occidentalis*.

Marx 116. Host deer; ditto; ditto.

Marx 117. Host ?; Santa Rosa, Cal.; ditto; ditto.

Marx 118. Host ?; California; ditto; ditto.

Marx 119. Host ?; Occidental, Cal.; ditto; ditto.

B. A. I. 2155. Host California deer; collected by Cooper Curtice; determined by Marx as *D. occidentalis*.

B. A. I. 3209. Host *Bos taurus*; Grand Island, Nebr.; collected by Hake & Co.; determined by Stiles in 1901 as *D. electus*. A male *D. occidentalis* mounted on slide with a female of another species.

B. A. I. 3512. Host *Bos taurus*; San Luis Obispo, Cal.; collected by G. F. Foulkner, June 2, 1903; determined by Ransom as *D. reticulatus*; redetermined by Stiles as *D. occidentalis*.

B. A. I. 3590. Host *Bos taurus*; San Luis Obispo, Cal.; collected by J. S. Jackson, February, 1904; determined by Ransom as *D. reticulatus*; redetermined by Stiles, 1909, as *D. occidentalis*.

U. S. P. H. & M. H. S. 9769. Host and locality unknown. Specimen came from the U. S. N. M. and bore the number 1348. To judge from the trochanter, this is *D. occidentalis*, but the capitulum and shape of the stigmal plate give rise to some doubt on this point.

For some months I considered this form identical with *D. andersoni*, and it is due to this fact that *D. occidentalis* has been quoted in medical literature as the species involved in transmitting Rocky Mountain spotted fever. As is shown in the present paper, however, the two forms are specifically distinct.

DERMACENTOR ANDERSONI Stiles, (1905) 1908.

(Figs. 9–13, 44–48, 55, 56, 74, 88, 89, 107, 108, 126, 127.

1905: *Dermacentor andersoni* Stiles, 1905g, 22, 24 (Bitter Root Valley, Montana); 1908m, 949.

1905: *Dermacentor occidentalis* of all writings on "Rocky Mountain Spotted Fever" as Ashburn, Craig, and Ricketts.

1908: *Dermacentor venustus* Marx [in part only], of Banks, 1908, 46–47.

Specific diagnosis. *Dermacentor* (p. 18): ♂, young and replete ♀: Caudal margin nearly or quite semicircular. Color grayish to red to deep reddish brown, or even nearly black (♀), except scutum; capitulum and legs lighter. Dorsum: Eyes not very prominent. Scutum well provided with whitish to greenish white rust, which varies considerably in different specimens; punctations large and small. Venter: Genital pore surrounded by numerous hairs. Anal ring nearly or quite circular, 0.27 to 0.35 mm. in diameter. Stigmal plates with prominent dorso-lateral prolongation, which shows a tendency to form at the caudal margin of the plate a right angle in the male and an acute angle in the female; aperture (pore, macula) and chamber large, elongate; goblets of medium size, usually from 17.5 to 32 μ in diameter, but may vary between 5 and 40 μ in diameter, are closely set and occupy nearly entire surface of plate except margin and terminal portion of elongation; meshes of middle layer about 9 μ in diameter; stem of goblets about 4.5 μ in diameter. Capitulum: Postero-lateral projections of base short. External article with a proximal, very large recurved tooth, a subapical smaller tooth, and a very small apical tooth. Hypostome, each half with a number of small subterminal denticles, followed by three rows of strong denticles, followed proximally by smaller denticles. Palpi, lateral margin convex, article 1 provided with four to five strong bristles on ventro-median margin; article 3 rather triangular dorsally, bluntly rounded distally, with several bristles on the concave margin of dorso-terminal portion and one or two terminal bristles; dorso-retrograde prolongation of article 2 (so prominent in *D. reticulatus*) nil, but one may be indicated on the postero-median angle. Legs: Lateral (outer) spur of coxæ I longer than the median (inner) spur; trochanter I provided with retrograde curved blade; terminal recurved spur not very well developed on tarsi I.

Male.—Length, 4 mm. (exclusive of hind legs; with legs outstretched, about 5 mm.); greatest breadth, 2.5 mm. Body oval, sides may be somewhat straighter than those of *D. salmoni*, more like those of *D. variegatus;* unmounted specimens 0.88 mm. broad at scapulæ (shoulders), specimens mounted under pressure measure up to 1.12 mm. at this point; the sides diverge from here till they reach their maximum divergence at coxæ IV, where the breadth may vary from 2.25 to 2.5 mm. in unmounted specimens to 2.9 or even 3.25 mm. in specimens mounted under pressure. Dorsum: Scutum covers entire dorsum (except capitulum); scapulæ project 0.27 to 0.30 mm. from border of excavation; cervical and marginal grooves distinct, cervical groove deeper anteriorly than posteriorly; scutum deep reddish brown, nearly covered with rust; pseudoscutum rather prominent, extending about 0.37 of length of scutum in median line; the most prominent red spots (namely, not covered with rust) back of pseudoscutum are: (*a*) Four elongate spots arranged in a semicircle and running radially, or nearly so, from caudal margin of the pseudoscutum; (*b*) two elongate to reniform spots back of these, one on each side of scutum; (*c*) an elongated median spot and two shorter, elongate, submedian spots corresponding to the same spots in *D. variegatus; (d)* but the forked spot usually so prominent in *D. salmoni* and *D. variegatus* is usually either exceedingly indistinct or absent; only a portion of the lateral margin of the scutum is white, and usually three or four small dark spots may be seen on each side; the rust on the eleven distinct postero-marginal festoons is quite variable, in some specimens rather profuse, in others very slight; numerous punctations of 8 to 32 μ in diameter, and a number of 64 μ or more in diameter, of less regular outline; very short, microscopic, whitish hairs issue from the center of at least some of the larger punctations. Foveolæ a short distance caudad of pseudoscutum, near antero-median corner of coxæ IV, and measuring about 80 μ in diameter. Venter: Surface sparsely beset with hairs, which, with the circular pores through which they pass, are noticeably more numerous around the genital pore. Genital pore between coxæ II. Stigmal plates 0.416 to 0.640 mm. long (through the aperture); breadth of plate (including prolongation), 0.480 to 0.656 mm.; goblets may attain 157 in number and 5 to 40 μ in diameter. Capitulum: 0.70 to 0.80 mm. long; base 0.528 to 0.72 mm. in breadth, and 0.288 to 0.352 mm. from posterior margin to anterior margin of portion over palpi; postero-lateral prolongations short, about 48 to 64 μ in length, rather blunt. Mandibles (chelicerae) 0.928 to 1.072 mm. long; digit 96 to 104 μ long; dorsal process of internal article elongate transversely and forming two transverse ridge-like edges directed toward the body, to some extent resembling the visors of two caps. Hypostome with seven to nine large denticles in each row. Palpi 480 to 640 μ long, each palpus 272 to 320 μ in maximum breadth; article 2 with six to seven bristles on ventro-median edge; article 3 with two to three bristles on ventro-median edge. Legs: Coxæ I to IV with curved mesial margin; coxæ IV present two types which grade into each other, and the two may even be found in one specimen; they may be relatively short or exceedingly long; ventral margins of legs bear spurs, especially femur and tibia IV; tarsi II to IV with large subterminal recurved spur.

Female. Dorsum: Scutum 1.56 to 1.62 mm. long in median line; 1.4 to 1.9 mm. broad at lateral angles; very prominent because of its whitish color; scapulæ project 0.256 to 0.352 mm. from border of excavation; from shoulders, the sides of scutum diverge in a rather irregular line to the lateral angles, then converge in nearly straight lines to the postero-lateral angles, when they suddenly change direction, converging much more rapidly, and forming a bluntly rounded posterior angle. Cervical grooves rather well developed, each forming an obtuse angle, at its equator, toward the median line, thus forming an hourglass like outline, deeper anteriorly than posteriorly; the distal, diverging portion of the hourglass is paralled each side by a more or less well developed dark line; eyes at lateral angles; large punctations somewhat more prominent antero-laterally; rust not so thick in antero-median field. Uncovered portion of margins as elsewhere, and absent from lateral angles. Uncovered portion of dorsum shows coarser and finer, also irregularly shaped punctations, a distinct median and two submedian grooves, and distinct marginal groove limiting the eleven postero-marginal festoons. Foveolæ 0.096 to 0.112 mm. in diameter, a short distance caudad of scutum. Venter: Punctations and fine hairs present; genital grooves run subparallel caudad, diverging slightly to a point about halfway between coxæ IV and anus, whence they diverge markedly toward lateral margin; median post-anal groove distinct in unmounted specimens. Stigmal plate 0.5 to 0.528 mm. long (through aperture); breadth (including prolongation) about the same; goblets may attain 120 in number and 12 to 32, occasionally 40 μ in diameter. Capitulum: 0.672 to 0.848 mm. long; base 0.60 to 0.736 mm. broad, 0. 24 to 0.288 mm. long from posterior margin to anterior margin of portion over the palpi; postero-lateral prolongation 16 to 32 μ, bluntly rounded; porose areas nearly meet in median line. Mandibles 0.96 to 1.118 mm. long; digit 96 to 128 μ long; dorsal process of internal article elongated somewhat transversely, with two pointed teeth. Smaller anterior denticles of hypostome confined to smaller area than in male; seven to nine or ten large denticles in each row. Palpi 0.544 to 0.672 mm. long by 0.256 to 0.32 mm. broad; article 2 with four to six strong bristles and article 3 with one to three bristles on ventro-median edge. Legs: Coxæ diverge II to IV; lateral (outer) spur of coxæ I may attain 320 μ in length; ventral margins of legs, especially femur and tibia IV, bear spurs or knobs or both; tarsi II to IV with two ventral chitinous knobs, showing some variation in different individuals.

Young female.—This seems to be the form most commonly found. Length 4.5 to 5 mm. (exclusive of hind legs; with hind legs extended may attain 6.5 to 7 mm.); greatest breadth 2.3 to 2.6 mm. Body oval, rather similar to male, but not quite so flat; unmounted specimens 0.712 to 1.0 mm. broad at scapulæ, specimens mounted under pressure may attain 0.875 to 1.062 mm. at this point; the sides diverge from here to a maximum breadth (2.312 to 2.6 mm. in unmounted and 2.31 to 3.18 mm. in specimens mounted under pressure) about at stigmal plate. Dorsum: Scutum covers about 0.43 of length of body (exclusive of head); eyes not very distinct in unmounted specimens, more distinct as translucent spot with radial structure in mounted, cleared specimens. Venter: Vulva between coxæ II.

Replete female.—May attain 16 mm. long by 9.5 mm. broad by 6 mm. thick. Form varies with degree of repletion; legs I are lateral of scutum so that anterior margin may reach 2.37 mm. in breadth; from here body increases rapidly in breadth, reaching a maximum in region of coxæ III to IV; a slight constriction is common near stigmal plates, from which point the breadth rapidly decreases. Dorsum: Eyes somewhat more distinct than in young female, owing to their more prominent position due to change in form of body. Venter: Vulva may shift to intercoxal space of legs I to II; a radial submedian groove may appear each side between anal and genital groove; otherwise similar to young female except for proportions due to greater repletion. Legs: Coxæ much farther apart than in young female, due to repletion.

Hexapod larva.—On several occasions replete female specimens were allowed to oviposit, and the eggs were allowed to hatch. Length of larva may attain 0.656 mm.; greatest breadth 0.316 mm.; the caudal portion of the body is broader than the anterior. Scutum 0.224 mm. long, scarcely emarginate anteriorly; a small light, but slightly indistinct, spot (apparently the eye) is visible at the lateral angle, and back of this the scutum rounds off very abruptly; accordingly, the scutum is entirely different from that of the adults. Cuticle of the body, except where covered by heavier chitinous structures, is provided with wrinkles, like striations, of slightly irregular course, but for the greater part running transversely. Posterior margin with nine distinct, and two less distinct festoons, each of which, except the median, bears both dorsally and ventrally, a small hair directed postero-mediad. Hairs are present also on other portions of the body and are arranged more or less symmetrically. Capitulum is decidedly *Rhipicephalus*-like in appearance, 176 μ broad, and its base is drawn out each side into a sharp lateral angle; postero-lateral prolongations (compare adults) are lacking, the proximal margin being straight and even. Mandibles 176 to 200 μ long; digit 22μ; external article with two distinct teeth, the anterior smaller, and the posterior larger; dorsal process of internal article difficult to analyze, apparently elongated transversely, and possessing two (or three??) teeth. Hypostome spatulate, each half with two longitudinal rows of distinct denticles, five to six in each row; proximally of these, there are several indistinct denticles. Palpi may or may not extend beyond hypostome; when straightened under pressure, however, they are slightly longer than hypostome; maximum breadth 44 μ; article one very small, scarcely visible; article two measures about 44 to 66 μ long, and bears several stout serrate bristles; one bristle is situated near distal end on ventro-median edge; one near proximal end on lateral border; one on dorso-median border near the middle of the article; and one dorsally near proximal end; article three measures 44 to 52 μ long, and bears several bristles, the most prominent of which are: one proximal, ventrally; one lateral, and two near the distal end; article four extends directly ventrad in a subterminal depression of article three, and bears about seven or more longer and shorter terminal bristles. Coxæ increase in size and are slightly divergent from I to III; coxæ I have a strong ventral spur directed caudad, and three prominent bristles, one of which is anterior and terminal, one on the median border, and one ventral near lateral border; the ventral spur of coxæ II to III is smaller than that of coxæ I; anterior and posterior lateral bristles on coxæ II and III corresponding to those of coxæ I, but the median bristle appears to be wanting; ventrally, on the body, there is a pair of bristles between the postero-median corners of coxæ I, II, and III. Four pairs of small stigmata are present, one stigmal opening being situated latero-caudad of each of coxæ I, one caudad of each of coxæ II and III, and one pair on transverse anal zone. The articles of the legs are well provided with bristles, rather symmetrically arranged, but no spurs are present. Pulvillum extends to about the middle of the claws. Anal ring 44 μ in diameter, and enclosing two lateral labia, each with a post-equatorial bristle.

Type. -U. S. P. H. & M. H. S. 9467.

Hosts.—Man (*Homo sapiens*), cattle (*Bos taurus*), horses (*Equus caballus*) dogs (*Canis familiaris*), rabbit (*Lepus* sp.), and apparently the ground squirrel or gopher (*Citellus columbianus*), and an undetermined species of squirrel.

Geographical distribution.—Montana, Washington State, Colorado, and (?) Idaho.

This species has passed through a varied and confusing history. Its variation is such that I have changed my opinion upon it at least a dozen times; in 1905 I determined it is as a new species, *D. andersoni*, but later in correspondence with various writers I suppressed the species in favor of *D. occidentalis*, thus adopting a determination made by Curtice some years ago. Marx confused the species with *D. americanus* (=*electus*). Very recently Banks (1908, 47) has identified it with *D. venustus* of Texas.

I have now been able to examine a large series of specimens and am persuaded that the recognition of a new species was justified. After finding certain characters, it now seems strange how it was possible to confuse it with other forms, yet in this confusion *D. andersoni* has simply repeated the history of many other species.

D. andersoni as described here is the common tick of the Bitter Root Valley, Montana, and is the form which has been collected by authors who have worked on Rocky Mountain Spotted Fever in that region. In all literature on that disease, this is apparently the tick[a] referred to under the name *Dermacentor occidentalis*.

In connection with the foregoing discussion I have compared the following specimens in the collection of the U. S. P. H. & M. H. S.:

9465. Host *Lepus* sp., at Polaris, Mont.; collected by Dr. F. M. Poindexter in 1904.

9466. From Victor, Mont.; collected by Dr. J. J. Buckley, May 28, 1904.

9467. Host *Equus caballus*, at Woodman, Mont.; collected by Mr. John Mills, May, 1904.

9468. From Polaris, Mont.; collected by Dr. F. M. Poindexter, 1904.

9470. From Missoula, Mont.; collected by Prof. M. J. Elrod, May 4, 1904.

9471. Host *Homo*, at Missoula, Mont.; May 10, 1904.

9472. From River View, Fork Valley, Mont.; collected by E. S. Hall.

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[a] I assume full responsibility for the erroneous use of the name *D. occidentalis* in medical literature, but have no apologies to make for the confusion, for it was made at a time when the two species could not be distinguished satisfactorily.

9473. Host *Homo*, in Montana; collected by Doctor Gates, April 30, 1904.
9474. From Woodman and Lo Lo, Mont.; collected by Mr. McGrath.
9475. Near Lo Lo, Mont.; collected by Mr. John Mills.
9476. Host *Homo*, at Missoula; collected by Dr. J. J. Buckley, May 12, 1904.
9477. From River View, Fork Valley, Mont.; collected by E. S. Hall, May, 1904.
9478. Host *Homo*, in foot hills south of Hamilton, Mont.; May, 1904.
9479. Host *Homo*, at Nimrod, Mont.; collected by Doctor Spottswood, May 16, 1904.
9480. Host ?, at Red Lodge, Mont.; collected by Mrs. J. Flaherty, May, 1904.
9481. From Foy's Lake, near Kalispell, Mont.; May, 1904.
9482. Host *Homo*, near Hamilton, Mont.; collected by Doctor McGrath, April and May, 1904.
9483. From Grantsdale, Mont.; collected by A. L. Holt, May, 1904.
9484. From Missoula, Mont.; collected by Mrs. C. W. Stiles.
9485. Host *Homo*, at Missoula, Mont.; collected by Doctor Spottswood, May 9, 1904.
9486. Host *Homo*, at Missoula, Mont.; collected by Doctor Spottswood, April 22, 1904.
9487. Host *Bos taurus*, near Hamilton, Mont.; collected by Doctor Tuttle, May 22, 1904.
9488. Host *Homo*, at Lo Lo, Mont.; collected by Doctor Gwinn.
9489. From Trail Creek, Mont.; May 23, 1904.
9490. Host *Homo*, at Missoula, Mont.; collected by Doctor Pixley, May, 1904.
9491. From Polaris, Mont.; collected by Miss Emma Saltine, May, 1904.
9492. Host *Homo*, at Missoula, Mont.; collected by Stiles, May 10–15, 1904.
9493. Host *Homo*, at Missoula, Mont.; collected by Dr. J. J. Buckley, May, 1904.
9494. Ditto.
9495. From Fort Logan, Mont.; collected by Max Sarter.
9496. Host *Equus caballus*, near Jefferson, Mont.; May, 1904.
9497. From Missoula, Mont.; collected by Prof. M. J. Elrod.
9499. Host *Bos taurus* at Alhambra, Mont.; collected by Mrs. H. F. Staph, May, 1904.
9500. Host *Homo*, near Helena, Mont.; collected by Dr. T. D. Tuttle, May 22, 1904.
9501. From Watson, Mont.; May, 1904.
9502. Host *Equus caballus*, at Livingston, Mont.; collected by Doctor Tuttle, May, 1904.
9503. From Lo Lo, Mont.; collected by John Mills.
9504. Bitter Root Valley, Mont.; collected by Doctor Spottswood.
9505. Host *Bos taurus*, at Livingston, Mont., May, 1904.
9506. From Grantsdale, Mont.; collected by A. L. Holt, May, 1904.
9507. Host *Equus caballus*, at Grants Creek, near Missoula, Mont.; collected by Dr. J. J. Buckley, May 9, 1904.
9508. Host *Equus caballus*, at Nelson Gulch, near Helena, Mont.; collected by Dr. T. D. Tuttle, May, 1904.
9509. Host *Homo*, at Grantsdale, Mont.; collected by A. L. Holt.
9510. Host *Equus caballus*, near Helena, Mont.; collected by Dr. T. D. Tuttle.
9511. Host *Homo*, at Helena, Mont.; collected by Dr. T. D. Tuttle, May, 1904.
9512. Host *Equus caballus*, near Florence, Mont.; collected by Ashburn and Stiles, May 19, 1904.
9513. From Dillon, Mont.; collected by Mr. Riley.
9517. From Fort Missoula, Mont.; collected by Ashburn and Stiles, May, 1904.
9518. Host *Bos taurus*, at Livingston, Mont.; collected by Dr. T. D. Tuttle, May, 1904.

9551. Hexapod larva, host *Citellus columbianus*. This is apparently the hexapod larva of *D. andersoni*.

9557. Ditto.

9589. From Montana, June, 1904.

9742. From Montana. In collection of Army Medical Museum.

9755. Belongs to U. S. National Museum, with two labels "24" and "25;" locality and host not given; belongs to *andersoni* group, perhaps to *D. andersoni*.

9760. Collected at Elko, Nev., by Mr. Whickham; belongs to *andersoni* group, possibly to *andersoni*.

9761. Collected by E. A. Bush, San Jose, Cal., August 2, 1887. Apparently *D. andersoni*, but not exactly typical specimen.

9768. From Utah. Material poor; belongs to *andersoni* group; if a *D. andersoni* it is not very typical.

9782. From U. S. National Museum, with label "*Dermacentor occidentalis* n. sp. Curtice, Easton, Wash. K." This is not *D. occidentalis*, but is closely related to, possibly identical with, *D. andersoni*.

10429. From Utah, collected by Alf. A. Robinson, 1908. Possibly represents a distinct species; it belongs to the *andersoni* group, but appears not to be a typical *D. andersoni*.

10018. Host *Equus caballus*, at Missoula; collected by Doctor King.

Marx 135. Host?; Glenwood Springs, Colo.; determined by Marx as *Dermacentor americanus* (= *electus*); determined by Neumann as *D. electus*. Shows exceedingly slight differences from the Montana forms of *andersoni*, but I do not feel justified on present material in describing it as distinct, although it is not impossible that examination of extensive material might reveal distinct differences

B. A. I. 3400: Host *Homo*, Wyoming; determined by Stiles in 1902 as *D. reticulatus*.

B. A. I. 2424: Host *Bos taurus*; Eagleville, Cal.; collected by C. H. Blemer, 1896; determined by Stiles and Hassall as *D. americanus*; redetermined by Stiles, 1909, as *D.? andersoni*.

The stigmal plates of this species, as found in the Bitter Root Valley, Montana, present the following characters:

The male plate (fig. 9) is similar to the female plate in general structure, but in the male the prolongation is at right angles to a plane drawn through the aperture, while in the female the prolongation forms an acute angle with that plane; 157 goblets are present.

Figure 10 shows the female plate of P. H. & M. H. S. 9503, taken at Lo Lo, Bitter Root Valley, not far from the point where the type (9467) was collected. The plate has a pronounced dorso-lateral subterminal prolongation. The aperture is relatively long. The plate measures 511 μ long, through the aperture, by 528 μ broad, including the prolongation. The goblets, 120 in number, occupy nearly the entire surface of the plate, except at the margins and near the end of the prolongation. On a very superficial focus these goblets come close together, and as a result they have a tendency to change their more or less circular outline for an angular outline; upon lowering the focus very slightly the appearance given in figure 9 is obtained.

The goblets are of medium size, may attain 32 or even 36 μ in diameter; the stems in the inner layer are seen more distinctly near the periphery than near the aperture.

DERMACENTOR VENUSTUS Marx, 1897.

(Figs. 14, 15, 57, 75, 90, 109.)

1897: *Dermacentor venustus* Marx in Neumann, 1897a, 365 (from Texas and New Mexico, not described) as syn. of *D. reticulatus* (Fabr.); 1901a, 345 (as a syn. of *D. reticulatus*).—Banks, 1908, 46–47 (in part only). —Salmon and Stiles, 1901a, 449 (as syn. of *D. reticulatus*).—Stiles, 1908m, 949.

Specific diagnosis.—*Dermacentor* (p. 18): ♂ and young ♀: Caudal margin about semicircular, anus nearer caudal than lateral margins. Color (alcohol specimens) dark reddish brown, except for rust on scutum; capitulum and legs lighter. Dorsum: Eyes not prominent. Scutum with a fair amount of whitish rust; punctations large and small. Venter: Genital pore of male surrounded by a number of punctations. Anal ring circular, prominent, 0.3 mm. in diameter. Stigmal plates with prominent dorso-lateral prolongation, the caudal margin of which forms with the plane of the aperture, a broadly rounded obtuse angle in the male and a right (?) angle in the female; aperture and chamber prominent; goblets medium size, attain about 10 to 43 μ, usually 24 to 40 μ in diameter, not thickly set in male, concentrated around aperture, more separated on prolongation (♂), attain 75 (♂) in number; meshes of middle layer 8 to 12 μ. Capitulum: Postero-lateral projections of base 64 μ long. External article (♂) with two teeth, one not very large proximal and one very small apical. Hypostome, each half with a number of minute subterminal denticles, followed by three rows of large denticles, followed by a number of scale-like denticles. Palpi, dorso-median margins convex; article 3 not distinctly triangular dorsally, appears somewhat quadrangular; a few subterminal bristles on median concave margin of dorsal portion of article 3. Legs: Lateral (outer) spur of coxæ I very slightly longer than the median spur; trochanter I ———; spurs or knobs on ventral margin of legs may be very prominent; tarsi I ———, tarsi II–IV ———; pulvillum ———.

Male.—Length 4.9 to 6.0 mm. long (exclusive of hind legs; with legs extended may attain 6.5 to 7.3 mm.); greatest breadth 2.93 to 3.75 mm. Body elongate oval; unmounted specimens attain 1.0 mm. broad at scapulæ, a specimen mounted under pressure measures 0.96 mm. at this point; the sides diverge from there in a slightly curved line to the broadest portion at coxæ IV, the curve being slightly interrupted at the eyes; the sides then converge rapidly. Dorsum: Scutum covers entire dorsum (except capitulum) or a slight marginal border may be uncovered; scapulæ project 0.24 to 0.48 mm. from rounded anterior border of excavation; cervical grooves very short, not very deep, only the usual cephalic portion being evident; marginal groove may be well marked; scutum reddish brown, with comparatively small, but fair, amount of whitish rust, and on account of small amount, the usual reddish spots are indistinct; pseudoscutum may be fairly well marked, though it is not prominent, extending about 0.40 of length of scutum in median line; small and large punctations present, the larger especially prominent. Foveolæ 80 μ in diameter, over coxæ IV. Venter: Surface with not unusually numerous hairs and punctations, but especially numerous near genital pore. Genital pore between coxæ II; genital groove present. Stigmal plate 0.48 mm. long (through aperture); breadth (including prolongation) 0.56 mm.; goblets may attain 75 in number and 12 to 43 μ in diameter. Capitulum: 0.896 mm. long; base 0.56 mm. broad, 0.32 mm. from posterior margin to anterior margin over palpi; postero-lateral prolongations 64 μ, blunt. Mandibles 0.88 mm. long; digit 128 μ long; dorsal process of internal article elongate transversely, with two visor-like ridges. Hypostome with seven to eight large denticles in each row. Palpi 480 μ long, each palpus 272 μ in maximum breadth; article 1 with five ventro-median bristles; article 2 with five such bristles; article 3 with (?) two such bristles. Legs: Coxæ I to IV with rather rounded mesial margin; coxæ IV quite large; ventral margins, especially of femur, tibia, and protarsus IV, with well developed spurs; at least tibiæ IV (others could not be studied) with large subterminal recurved spur.

Female.[a]—Dorsum: Scutum 1.52 mm. long in median line; 1.79 mm. broad at lateral angles; rather prominent because of its whitish rust; scapulæ project (?) ——; from shoulders the sides diverge in a convex line to lateral angles, then they converge in nearly a straight line to postero-lateral angles, when they converge more rapidly to form a bluntly rounded caudal angle. Cervical grooves very prominent, hour-glass shaped, deeper cephalad than caudad; eyes rather prominent, at lateral angles; large and small punctations present, the larger especially prominent in lateral fields; rust not so thick in antero-median field as elsewhere. Uncovered portion of dorsum with not very prominent hairs and punctations, a median and two submedian grooves present, marginal groove and postero-lateral festoons not very prominent. Foveolæ a short distance caudad of scutum, about 64 μ in diameter. Venter: Punctations and hairs not prominent; genital grooves diverge gradually to a point about halfway between coxæ IV and anus, then they diverge markedly toward lateral margin; median postanal groove distinct. Stigmal plate with prominent dorso-lateral prolongation which is at nearly right angle to a line drawn through the rather elongate aperture; about 105 goblets are present, attaining 10 to 30, even 43 μ in diameter. Capitulum: Something over 0.576 mm. long; base 0.672 mm. broad; porose areas rather deep, do not meet in median line, diverge anteriorly. Mandibles (?); digit (?); dorsal process of internal article (?). Smaller denticles on hypostome rather numerous; eight or nine large teeth in each row. Palpi (?); article 1 apparently with four, article 2 with five, article 3 with three ventro-median brisales. Legs: Coxæ diverge I to IV; spurs or knobs (?).

Young female.—Length 5.3 mm. (exclusive of hind legs); greatest breadth 3.3 mm. Body rather oval; unmounted specimen measures 1.0 mm. broad at scapulæ; the sides diverge from here in a convex line (slightly interrupted between legs I and II) to a maximum breadth at the stigmal plates. Dorsum: Scutum covers about 0.32 of the length of the body (exclusive of capitulum); eyes rather large and distinct. Venter: Vulva between coxæ II.

Type.—Marx 122, in U. S. National Museum.

Host.—Sheep (Ovis aries) in Texas.

In the Marx collection I find three bottles containing ticks which Marx determined as *Dermacentor venustus*, namely:

Marx 120. Host (?) ——: Las Cruces, N. Mex., one male; determined by Marx as *D. venustus*; determined by Neumann as *D. reticulatus occidentalis*. This is a member of the *andersoni* group, but it is not *D. occidentalis*. It may be *venustus*, but as there is only one specimen, which can not be mounted, I reserve judgment.

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[a] Only one specimen, which could not be mounted.

Marx 121. Host Mountain goat; Soldier, Idaho; one male determined by Marx as *D. venustus;* determined by Neumann as *D. reticulatus occidentalis.* Not being able to mount this, as it is a single specimen, I hesitate to make a definite determination; but it is not *D. occidentalis;* it bears a striking resemblance to *D. andersoni*, so far as can be seen on unmounted material.

Marx 122. Host *Ovis aries;* Texas; three males, one young female; determined by Marx as *D. venustus;* determined by Neumann as *D. reticulatus occidentalis.* This lot, I assume, is type of *D. venustus.*

Microscopic examination of the stigmal plates shows that they are quite distinct from *D. reticulatus*, but very closely related to *D. parumapertus* and *D. occidentalis.*

Figure 14 shows the plate of the male, which is seen to have a pronounced dorsal prolongation. The aperture is broad, but not very long, and lies slightly antero-mediad of the center of the body of the plate. The goblets are of medium size, circular to elongate, and attaining a diameter of 48 by 28 μ. They may attain 75 in number and are somewhat scattered; the goblets near the aperture are larger, those nearer the periphery are smaller and more scattered, those in the prolongation are smallest and the most scattered. The longitudinal diameter of the plate is 480 μ, the transverse diameter (including prolongation) is 544 μ.

The specific description of *D. venustus* as given by Banks is influenced by the fact that he includes *D. andersoni* in this species.

Mr. Banks (1908) has examined the types and drawings of *D. andersoni*, my drawings of Marx's *D. venustus*, and also Marx's original material, and apparently Marx's manuscript of *D. venustus.* He considered *venustus* and *andersoni* specifically identical and has published them as such under the name *D. venustus*, although he does not refer to the name *D. andersoni.* He says:

> Specimens come from various places in the West; Olympia, Yakima, Klikitat Valley, and Grand Coulee, Wash.; Fort Collins and Boulder, Colo.; Pecos and Las Cruces, N. Mex.; Bozeman, Mont.; Bridger Basin, Utah; Soldier, Idaho; and Texas (on sheep).
>
> This species is quite common in the Northwest. It has been included in *D. occidentalis* by Neumann, but was separated out by Doctor Marx in manuscript under the name I have adopted. It is larger than *D. occidentalis*, with more red and less white in the coloring, and differs in many minor points of structure, as size of porose areas, size of hind coxæ in male, etc. This is the species supposed to be concerned in the transmission of spotted fever in Montana.

Bank's description of *D. venustus* is based largely, and his drawings exclusively, on material which is in reality *D. andersoni.* This combination of circumstances gives rise to some complication. My interpretation of the best way to solve the difficulties is this: Marx's material and Marx's name are, as admitted by Banks, a part of *D. venustus* as described under Marx's manuscript name *D. venustus*, which is adopted by Banks, although Banks writes "n. sp." after this name. I can not assume that the type specimens of this *D. venustus* can be other than the Marx material, for any such assumption would necessarily carry with it a corollary that Banks had examined the unpublished manuscript and drawings of two personal friends (one deceased) and had taken advantage of these circumstances. It will be noticed that he did not mention the museum number of the type specimen of *D. venustus* as construed by him, and the most natural assumption is that it is to be found in Marx's material. Further, this interpretation preserves two specific names now published for their respective species.

If it were to be construed that the type of the species *D. venustus* as interpreted by Banks were not in Marx's material, then the type of *D. andersoni* examined by Banks would come up for consideration. To interpret this as type would produce confusion, and seems not only unnecessary but perhaps unwarranted. The only remaining possibility would be that the type of *D. venustus* is to be found in Banks's specimens (other than what he examined in my laboratory), but it is found impossible to follow this side of the question further, as it would be a reflection upon my friend Banks and would not simplify matters. If any person should be inclined to differ with me in this interpretation, it may be recalled that it is clear that the material of Banks's *D. venustus* would under any other interpretation include three distinct sets of type material, and it is equally clear that Marx's type material is the oldest.

The entire question at issue contains elements which are not covered in detail by the International Code.

DERMACENTOR PARUMAPERTUS Neumann, 1901.

(Fig. 16.)

1901: *Dermacentor parumapertus* Neumann, 1901a, 267–268 (host unknown, at Lakeside, Cal.).—Banks, 1907, 608; 1908, 45–46, pl. 8, figs. 8, 10.

1905: *Dermacentor electus parumapertus* (Neumann, 1901) Neumann, 1905d, 236.

The specific diagnosis as given by Neumann is as follows:

Male.—Unknown.

Female.—Body oval, swollen, slightly broader anteriorly, sides subrectilinear, 9 mm. long, 5.5 mm. broad. Color deep chestnut brown. Shield oval, slightly longer (1.5 mm. long) than broad, contour slightly sinuous back of eyes; eyes flat, blackish, large, near equator of shield; cervical grooves very broad, being confounded with lateral grooves; punctations numerous, unequal, the larger occupying especially the grooves and forming two longitudinal series on the median field; color deep chestnut-brown. Dorsal and ventral surfaces smooth, with numerous fine punctations. Vulva very small, very anterior, in plane of second intercoxal space. Anus very small, toward posterior third; no anal groove.

Peritremes (stigmal plates) very small (340 μ), oval, with a short retrodorsal prolongation. Rostrum small, 0.7 mm.; base short, at least twice as broad as long, rectangular, posterior angles slightly salient; porose areas small, deep, oval, subparallel. Hypostome spatulate, slightly rounded at extremity, with three rows of seven to nine teeth each side. Palpi thin, sides parallel; article 2 one and one-half times as long as article 3. Legs medium. Coxæ I divided into two short spines, the median thicker; a small spine at postero-external angles of coxæ II–IV. Tarsi progressively attenuated, terminated by a short spine.

Banks (1908, 45–46) restudied this form, which he describes as follows:

DERMACENTOR PARUMAPERTUS Neumann.

Male.—Dark red-brown, legs a trifle paler, no white markings, except sometimes a few small spots, and a minute white spot at tips of some joints of the legs. Capitulum moderately broad, hind angles only very slightly produced; palpi very short, not as long as width of capitulum; dorsum one and two-thirds times as long as broad, with many scattered, deep, but not very large punctures, submarginal furrow very distinct on the sides, less so behind; twelve impressed lines near posterior margin. Coxæ spined as usual, hind coxæ barely wider on base than long, legs rather short, hind pair not so much larger than the others, and the teeth below small and indistinct. Stigmal plate elongate, attenuate behind, the fore part around peritreme with large granules, a few down on the narrow portion, which is covered with smaller granules.

Length of male, 2.8 mm.

Female.—Shield and capitulum dark red-brown or almost black, without marks; abdomen blackish; legs red-brown, a faint white mark at tips of some of the joints. Capitulum moderately broad, hind angles distinctly prolonged behind, porose areas rather small, nearly circular, and well separated; palpi as long as width of capitulum. Shield plainly a little longer than broad, with many deep punctures; those in the depressed area each side especially large and numerous, almost confluent. Legs rather small and short; coxæ armed as usual. Stigmal plate small, with a distinct, although short and broad, dorsal prolongation; most of the surface with rather large granules, but those on the prolongation very small.

Length of female shield, 1.1 mm.

Specimens are from Lakeside, Cal. (also Neumann's type in the Marx collection), taken on man, and in a chicken house.

Distinguished from other forms most readily by lack of white on shield, by porose areas, and stigmal plate. After describing this species, Neumann later made it a variety of *D. electus* (*variabilis*), but it differs in many important characters from that species, and the granulations of the stigmal plate are much larger.

DERMACENTOR PARUMAPERTUS var. MARGINATUS.

This form agrees in general with the true *D. parumapertus*, but differs in several minor points. The posterior border of the female shield is margined with white; the porose areas are larger and rather closer together; the lateral lobes of the shield have fewer punctures, and the shield is more contracted behind the eyes; the stigmal plate of the female has a narrower dorsal prolongation, and the inner margin is more convex; the posterior angles of the capitulum are less prominent. Otherwise it is very similar to the type.

Several specimens from Mesa City, Ariz., from a jack rabbit (Cordley).

My material, apparently the type specimen, does not permit a detailed study.

A microscopic examination of the stigmal plates of the type specimens (Marx 143) shows that this form is quite distinct from *D. electus* and that it is closely related to *D. venustus* and *D. andersoni.* The slide of the type is not especially good, but certain features can be clearly recognized.

Figure 16 represents the stigmal plate of the female. It will be noticed that the plate is relatively small, the aperture, *and especially the chamber*, relatively very large; a prominent dorso-lateral prolongation is present; and the goblets are rather numerous (90) and of medium size, 7 to 25 μ in diameter. One of the most prominent features is the convexity of the surface, especially near the aperture. This convexity gives to the plate an appearance which is quite different from that noticed in the other species, where it may be relatively flat, or even concave. Two other characters of the plate are quite prominent: The goblets are unusually circular in outline and arranged rather regularly near the aperture; further, on the proximal border of the dorso-lateral prolongation there is a prominent, solid, broad margin, free of goblets or other similar structures.

Very closely allied to *D. parumapertus* is the following form:

DERMACENTOR PARUMAPERTUS MARGINATUS Banks, 1908.

(Figs. 17–19, 58, 59, 76, 91, 92, 110, 111, 128.)

1908: *Dermacentor parumapertus marginatus* Banks, 1908, 46, pl. 8, fig. 6 (host, Jack rabbit [probably *Lepus cremicus*]; Mesa City, Ariz.).

Banks has recognized as a subspecies of *D. parumapertus* a form taken from the Jack rabbit in Arizona. Owing to paucity of material of the type form, I am not altogether clear at present as to the relations of the two ticks in question. The following description is based upon material which is evidently Banks' *D. p. marginatus:*

SUBSPECIFIC DIAGNOSIS.—*Dermacentor* (p. 18): ♂ and ♀: Caudal margin practically semicircular, but anus nearer caudal than lateral margins, except in replete female. Color reddish brown, very little rust present (alcohol specimens); capitulum about the same color, but legs lighter. Dorsum: Eyes not very prominent, but quite distinct in mounted specimens. Scutum with only a slight amount of rust; large punctations much more prominent than the smaller. Venter: Genital pore surrounded by numerous large and small punctations, especially in male. Anal ring circular, 224 to 230 μ in diameter. Stigmal plates with prominent, broad, dorso-lateral prolongation; goblets of medium size, 7 to 32 μ in diameter, attain 44 to 99 in number, unusually circular, concentrated around aperture, but scattered on prolongation which forms caudally an obtuse angle in the male and an acute angle in the female; meshes of middle layer vary in diameter. Capitulum: Postero-lateral prolongations of base short. Hypostome, each half with a number of minute terminal and subterminal denticles, followed by three rows of larger denticles, followed by scales. Palpi, lateral margins convex; median dorsal margin prominently convex. Legs: The lateral (outer) spur of coxae I distinctly longer than the median which is quite short; trochanter I with a dorso-terminal retrograde blade, similar to *D. andersoni;* ventral knobs not well developed; tarsi I with poorly developed terminal spur.

Male.—Length 2.8 to 3.187 mm. (exclusive of hind legs; with hind legs extended may attain 4.18 mm.); greatest breadth 1.68 to 1.81 mm. Body rather triangular; unmounted specimens attain 0.64 to 0.688 mm. broad at scapulæ; specimens mounted under pressure may attain 0.75 mm. at this point; the sides diverge from here in almost a straight line until they reach their maximum diameter. Dorsum: Scutum covers nearly or quite entire dorsum (except capitulum); scapulæ project 0.08 to 0.16 mm. from border of excavation, which is rather convex cephalad; cervical grooves deep and prominent anteriorly; marginal groove distinct; scutum dark reddish brown, very little rust present (alcohol specimens); pseudoscutum very indistinct; numerous fine punctations and hairs present; also large punctations, which are unusually prominent, and more apparent in marginal field than elsewhere; eyes well marked at coxæ II. Foveolæ just back of plane of anterior margin of coxæ IV, 64 μ in diameter. Venter: Ventral surface with coarse and fine punctations and large and small hairs, especially numerous near genital pore; four longitudinal rows of large hairs especially prominent arranged near median and lateral margins of coxæ. Genital pore between coxæ II; genital groove distinct. Stigmal plate rather small and rather similar to *D. occidentalis;* 0.256 to 0.288 mm. long (through relatively large aperture); 0.32 to 0.40 mm. broad (including long slender dorsolateral prolongation); attaining 0.488 mm. when measured from antero-median margin to tip of prolongation; goblets may attain 44 in number, 13 to 32 μ in diameter, rather concentrated around aperture, fewer and more scattered on prolongation. Capitulum: 0.56 to 0.6 mm. long; base 0.432 to 0.512 mm. broad by 0.192 mm. from posterior margin to anterior margin over palpi; postero-lateral angles project 32 to 40 μ, rather blunt. Mandibles 0.7 to 0.8 mm. long; digit 88 μ long; external article with large proximal and smaller distal tooth, the usual minute apical tooth not visible (absent?); dorsal process of internal article not very prominent, elongate transversely in two visor-like ridges. Hypostome with minute subterminal denticles, followed by three rows of six to eight large denticles, followed by scale-like denticles disappearing median to lateral. Palpi 0.368 to 0.4 mm. long; each palpus about 144 to 200 μ in maximum breadth; article 1 small, with two bristles, article 2 with four bristles, article 3 with two bristles. Legs: Coxæ IV rather large, may attain plane of caudal margin of stigmal plates; ventral knobs not well developed; tarsi I with poorly developed terminal spur; tarsi II–IV with poorly developed subterminal and not very well developed terminal spur.

Female.—Dorsum: Scutum 1.23 to 1.37 mm. long in median line; 1.5 to 1.6 mm. broad at lateral angles; distinct despite small amount of rust present (alcohol specimens); scapulæ project 160 to 270 μ from anterior margin; from shoulders the sides of scutum diverge in a very convex line to lateral angles, from here they converge in a line (which may be slightly concave) to the blunt postero-lateral angles, then they converge more rapidly forming a bluntly rounded caudal margin; in general the outline appears oval. Cervical grooves very distinct, broad caudally, forming an hourglass. Eyes at lateral angles, not prominent under a hand lens, but may appear light, more prominent, and rather salient under the microscope. Some fine punctations present; larger punctations quite prominent. Rust appears on postero-lateral margins. Uncovered portion of dorsum with numerous not very prominent punctations and hairs; also a distinct median and two submedian grooves, marginal groove not very prominent. Venter: Fine and coarse punctations (cuticular rings with hairs) present; one row of hairs especially prominent each side, lateral of lateral spine of coxæ; genital grooves diverge slightly to a point about half way between coxæ IV and anus, whence they diverge markedly toward lateral margin; median postanal groove present. Stigmal plates 0.384 to 0.4 mm. long (through unusually large aperture and chamber); 0.352 to 0.384 mm. broad, including prominent projection; goblets 73 to 99 in number, 7 to 30 μ in diameter. Capitulum: 0.83 to 0.94 mm. long; base 0.656 to 0.72 mm. broad, 0.24 to 0.27 mm. from posterior margin to anterior margin over palpi; postero-lateral angles short, 16 to 48 μ, bluntly rounded. Porose areas nearly circular to rather oval, do not meet in median line. Mandibles attain 1.12 mm. long; digit 128 μ long; external article with three teeth, proximal large, subapical smaller, apical very small; dorsal process of internal article apparently with two prongs. Hypostome with smaller terminal denticles, followed by three rows of large denticles, ten to twelve in each row, disappearing median to lateral. Each palpus attains 0.59 to 0.64 mm. long, by 0.256 mm. broad; article 1 triangular to nearly quadrangular ventrally, with four ventro-median bristles; article 2 much longer than broad, with five ventro-median bristles; article 3 nearly quadrangular dorsally, with two to three ventro-median bristles; article 4 small, ventro-subterminal of article 3 and with several bristles. Coxæ II to IV diverge; median spur on coxæ I unusually short when compared with lateral spur, which attains 160 μ in length and shows a tendency to curve laterad; tarsi I with very poorly developed terminal spur; spur on tarsi II to IV slender, not very prominent.

Young female.—Length 3.375 to 3.875 mm. long (exclusive of hind legs; with hind legs extended attains 5.0 to 5.3 mm.); greatest breadth 2.0 to 2.625 mm. Body elongate oval to broad oval; unmounted specimens 0.8 to 0.88 mm. broad at scapulæ; the sides diverge from here in a markedly convex line (which is slightly interrupted between legs I and II) to their maximum, when they converge rapidly, forming a bluntly rounded caudal margin. Dorsum: Scutum covers 0.43 to 0.50 of length of body (exclusive of head); postero-marginal festoons evident but not unusually prominent. Foveolæ a short distance caudad of scutum, 64 to 112 μ in diameter. Venter: Vulva between coxæ II. Anus nearer caudal than lateral margins.

Replete female.—May attain 10.3 mm. long by 7 mm. broad by 5 mm. thick. Form oval. Legs I lateral of scutum so that anterior margin of body (back of head) seems almost straight and attains 1.84 mm. in breadth; from here body increases rapidly in breadth to its maximum in the region of coxæ IV; no constriction visible at stigmal plate; caudal end very bluntly rounded. Dorsum: Eyes slightly more distant than in young female, owing to their more prominent position, due to change in form of body. Scutum with rust at margins as in young female. Median and submedian grooves visible as lines, and a pair of broken grooves (indicated as lines) lateral of submedian grooves. Venter: Vulva has shifted to anterior of plane of coxæ II. Genital groove still distinct, postanal groove appears merely as a line. Legs: Coxæ much farther apart, due to repletion.

TYPE. Collection, Banks, Washington, D. C.

Of this form I have seen the following specimens:

Marx 137. Host, Jack rabbit; Fort Bowie, Ariz.; determined by Neumann as *Dermacentor electus*.

Marx 139. Host, Jack rabbit; Fort Bowie, Ariz.; determined by Neumann as *Dermacentor electus*.

? Marx 142. Host and locality ?; determined by Neumann as *Dermacentor electus*. This is a member of the *andersoni* group, and is probably the same as 137, but the material is not very favorable for identification.

B. A. I. 3415. Host, *Lepus campestris;* Deming, N. Mex.; collected by A. H. Wallace; determined by Stiles in 1902 as *D. reticulatus.*

Marx 129. Host (?), Elko, Mont.; determined by Marx as *D. americanus;* determined by Neumann as *D. electus;* two gravid females, not especially favorable for determination; clearly belonging to *andersoni* group, and apparently to *D. parumapertus marginatus.*

Figures 17 to 19 show the male and female plates of a tick which Neumann considered *D. electus.* Microscopic examination of the plates, however, shows that we are dealing with specimens of the *Andersoni* group, for the goblets are of medium size. In the male (fig. 17) the plate resembles that of the male *D. venustus* and *D. occidentalis* to some extent; in the female, the plate reminds one strongly of that of *D. parumapertus.* The convexity of the plate, the arrangement and the circular form of the goblets, the very large aperture and chamber, and the very broad anterior margin (*m*) of the prolongation clearly distinguish this form from all others forms discussed in this paper, with the possible exception of *D. parumapertus.* It is desirable to study a number of specimens of the latter in order to decide upon the relation which these two forms bear to each other.

SALMONI GROUP.

We may next pass to three species (*D. nigrolineatus*, *D. salmoni*, and *D. variegatus* Neumann [= *D. albipictus* Packard, teste Banks], which by means of their stigmal plates can be easily distinguished from all other species of *Dermacentor* discussed in this paper; it is more difficult to distinguish *salmoni* from *variegatus* by this means, but the latter is very pilose and of different outline from *salmoni.*

The goblets in these species are large, namely, usually 30 to 85 μ in diameter, and hence relatively few (56–126) in number when compared with the *reticulatus* group.

DERMACENTOR NIGROLINEATUS (Packard 1869) Banks, 1907.

(Figs. 20–25, 60–64, 77, 78, 93, 94, 112, 113, 129, 130.)

1869: *Ixodes nigrolineatus* Packard, 1869b, 66 (on *Cervus virginianus*, Northern New York).

1907: *Dermacentor nigrolineatus* (Packard, 1869) Banks, 1907, 608.

Specific diagnosis.—*Dermacentor* (p. 18): ♂ and young ♀: Caudal margin semicircular. Color (alcohol specimens) dirty yellow to reddish brown; capitulum and legs may be either lighter or darker than body and scutum. Dorsum: Eyes rather distinct, salient in female, less prominent in male. Scutum without rust, punctations small, with short hairs. Venter: Genital pore surrounded by hairs (♂) which are much less numerous and less prominent in female; entire cuticle quite pilose, much like *D. variegatus.* Anal ring nearly circular, slightly broader than long, transverse diameter (mounted specimens) about 252 μ, sagittal diameter 240 μ. Stigmal plates without any dorso-lateral prolongation; aperture relatively small, preequatorial in male, less distinctly so in female; goblets rather large, 15 to 53 μ in diameter, more numerous in postequatorial than in preequatorial portion. Capitulum: Postero-lateral projections prominent, about 80 μ long. Hypostome, each half with a number of minute subterminal denticles, followed proximally by three rows of six to eight large denticles, followed by several smaller scale-like denticles. Palpi, both lateral and median margins convex; two to three bristles on ventro-median margin of article 1; three to five on same margin of article 2; two to three on same margin of article 3; article 3 rather triangular, in dorsal view, its distal margin somewhat rounded; dorso-retrograde prolongation at postero-median angle of article 2 may be indicated, but is insignificant if present. Legs: Lateral (outer) spur on coxæ I somewhat narrower than median (inner) spur, but of about same length; trochanter I with prominent sharp retrograde spine-like distal spur; ventral margins of femur IV, tibia III-IV and protarsus IV may bear slight spurs or knobs, but these if present are not at all prominent; tarsi I with small terminal spur.

Male.—Length, 3.36 to 4.0 mm. long (exclusive of hind legs); greatest breadth, 1.99 to 2.5 mm. Body oval; unmounted specimens attain 0.88 mm. broad at scapulæ; the sides diverge from here until they reach their maximum breadth at coxæ IV or at stigmal plates. Dorsum: Scutum covers nearly entire dorsum (except capitulum and caudal margin); scapulæ project about 0.208 mm. from border of excavation; cervical grooves not prominent, more distinct anteriorly than posteriorly; marginal groove not distinct, except at sides in some specimens; scutum dirty yellow to brownish, without rust; pseudoscutum very indistinct (one specimen) or absent; eleven postero-marginal festoons, the median may be distinctly narrower than the others; numerous minute punctations with short hairs present. Foveolæ near median margin of coxæ IV, may attain 64 to 80 μ in diameter. Venter: Ventral surface thickly beset with hairs. Genital pore between coxæ II; genital groove distinct, diverging rapidly caudad of coxæ IV. Stigmal plate 0.455 to 0.546 mm. long, 0.224 to 0.336 mm. broad; goblets about 68 in number and 15 to 53 μ in diameter. Capitulum: Small, may attain about 0.504 mm. in length; base 0.464 to 0.48 mm. broad, 0.20 mm. long from posterior margin to anterior margin of portion over palpi; postero-lateral prolongations about 80 μ long. Mandibles about 0.656 to 0.704 mm. long; digit attains 120 μ in length; external article with rather large recurved proximal tooth (smaller subapical tooth not observed), and very minute apical tooth; dorsal process of internal article elongate transversely with two visor-like transverse ridges. Hypostome with six to eight large denticles in each row. Palpi 0.320 to 0.368 mm. long, each palpus attains 0.184 to 0.224 mm. in maximum breadth; article 2 with four, article 3 with two to three bristles on ventro-median edge. Legs: Coxæ, lateral spurs of II to IV prominent, median spurs of II to IV small or absent; tibia III and femur, tibia, and protarsus IV with or without slightly developed knobs or spurs; tarsi I with small or fairly well developed terminal spur, tarsi II to III with subterminal and terminal spurs, tarsi IV apparently with terminal spur only.

Young female.[a]—Length 5 to 6.6 mm. (exclusive of hind legs); greatest breadth 3 to 3.3 (unmounted) to 4.99 mm. (mounted). Body rather elongate oval; unmounted specimens attain about 0.80 to 0.96 mm. broad at scapulæ, specimens mounted under pressure attained only a slightly greater breadth, 0.992 mm.; the sides diverge from here to a maximum breadth about at coxæ III to IV, then they may run nearly parallel for a short distance or converge at first gradually, then more rapidly to bluntly rounded caudal margin. Dorsum: Scutum covers nearly 0.32 of body length (one specimen) (exclusive of capitulum); 1.12 to 1.44 mm. long in median line; 0.992 to 1.312 mm. broad at lateral angles; rust absent; scapulæ project 0.176 mm. from border of excavation; from shoulders, the sides of the scutum curve (convexity laterad) to the lateral angles, then converge with somewhat sigmoid course to more or less pointed posterior angle. Cervical grooves distinctly deeper cephalad than caudad, each forming mediad the concave side of an hourglass. Punctations small, with fine hairs, but in unmounted material a few large punctations also are seen. Eyes rather salient, at lateral angles, very dark in unmounted, translucent in cleared specimen. Uncovered portion of dorsum shows numerous fine punctations (circular pores) with fine hairs; also several longitudinal grooves (which can not be safely interpreted because of shrunken condition of specimens). Foveolæ 32 μ in diameter, between coxæ IV. Venter: Punctations and fine hairs present, but not so numerous as in male; genital grooves diverge gradually, then form curve (convexity mediad) caudad of zone of coxæ IV, then diverge rapidly latero-caudad; median postanal groove apparently present (material poor). Stigmal plate relatively much broader and shorter than in male, 392 μ long by 308 to 378 μ broad; goblets attain about 103 in number, usually 24 to 52 by 24 to 40 μ in diameter, extremes 17 and 52 μ. Vulva in zone between coxæ I and II. Capitulum (only one good specimen): About 0.72 mm. long; base 0.592 mm. broad, and 0.282 mm. long from posterior margin to anterior margin of portion over palpi; postero-lateral angles 48 to 64 μ long, rather blunt; porose areas broad, do not meet in median line. Mandibles about 0.88 mm. long; digit 120 μ long; external article with large proximal recurved tooth, smaller subapical and very small apical tooth; dorsal process of internal article with two teeth. Hypostome with numerous minute subterminal denticles, followed by three rows of larger denticles on each half, ten or more in each row, disappearing first from median, then intermediate, lastly from external row. Palpi 0.416 to 0.464 mm. long; each palpus may reach a maximum of 0.192 to 0.224 mm. in breadth; article 2 may have five, article 3 may have three bristles on ventro-median margin. Legs: Coxæ I to IV diverge gradually away from median line; coxæ I and trochanter I about as in male; knobs or spurs on tibia III, femur, tibia, and protarsus IV not prominent; tarsi I with fairly well developed terminal spur; tarsi II to IV with terminal spur and two small subterminal knobs.

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[a] Specimens poorly preserved as to form.

Replete female.—Not seen.

Nymphal skin.—Stigmal plate with 14 goblets (fig. 25).

TYPE.—Museum of Comparative Zoology, Cambridge, Mass.

HOSTS.—Deer (New York, Wisconsin, Texas), *Equus caballus* (Oklahoma, Tennessee).

The specific diagnosis as given by Banks (1908, 48–49) reads as follows:

Male.—Rather pale red-brown, no white markings, but the black cæcal marks show through in most specimens as several irregular lines behind; legs more yellow-brown. Capitulum small and narrow, its posterior angles produced into long spines; palpi very small and stout. Dorsum slender, about one and two-thirds times as long as broad; middle anterior region smooth and shining, sides and behind densely punctured, and with many short hairs; lateral furrows not very distinct, twelve impressed lines behind, but the festoons are not as obvious as usual. Legs rather short, coxæ with usual spines, coxa IV but little wider at base than long; stigmal plate large, elliptical, without dorsal prolongation, and covered with many large granules.

Length of male, 3.5 mm.

Female.—Shield red-brown, without marks; legs similar; abdomen dark red-brown. Capitulum scarcely twice as broad as long; hind angles distinctly prolonged behind; porose areas large, oval, and distinctly separated; palpi small and short, not as long as width of capitulum. Shield plainly longer than broad, broadest much before the middle, tapering and almost pointed behind, with very few punctures. Legs small and short, the tarsi very short; coxæ with the usual spines, that on IV no longer than on III. Stigmal plate elliptical, of same shape as in male, no dorsal prolongation, and covered with many large granules.

Length of female shield, 1.2 mm.

Through the kindness of Mr. Nathan Banks I have been able to examine a male tick (U. S. P. H. & M. H. S. 10592) determined by him as *Dermacentor nigrolineatus*, host and locality not stated. This measures 3.46 mm. long by 1.99 mm. in greatest breadth.

The two stigmal plates (fig. 20) measure, respectively, 0.455 mm. long by 0.280 mm. broad, and 0.511 mm. long by 0.224 mm. broad. The outline of the plate is quite regular, without any indication of a dorso-lateral prolongation. There are 69 goblets present; these are circular to oval, 32 to 40 μ in diameter, and are more numerous in the postequatorial portion of the plate. The macula or pore is in the preequatorial portion of the plate.

Of other characters, the following may be mentioned:

The base of the capitulum (fig. 60) is 0.448 mm. broad; the postero-lateral angles project about 96 μ; the palpi are about 320 μ. Trochanter I has a well-developed sharp dorso-distal retrograde spur. The venter is thickly beset with hairs, and hairs and pores are numerous around the male genital pore.

A male of U. S. P. H. & M. H. S. 9992 (mounted) appears to be specifically identical with Banks's specimen. It was collected in New York. According to our catalogue record it was determined by Banks as *D. variegatus*.

It measures 3.36 mm. long by 2.33 mm. broad; the stigmal plates measure 0.518 by 0.322 and 0.546 by 0.336 mm.

There is a single female specimen of 9992. Unfortunately it is damaged. Its scutum (fig. 78) measures 1.34 mm. long; the stigmal plates are less elongate than in the male; they measure 0.392 by 0.308 mm. and 0.392 by 0.378 mm. There are 66 goblets present, which measure 22 to 42 μ in diameter. The injured body measures (mounted) 6.6 mm. long by 4.99 mm. broad, without the capitulum, which is lost.

B. A. I. No. 4288 contains three female ticks collected from *Equus caballus* in Davidson County, Tenn., by J. C. Drake, January 11, 1906. Two unmounted specimens measure 5.75 to 7.5 mm. long. One mounted specimen measured shows a scutum 1.46 mm. long. The stigmal plates measure 0.490 by 0.420 and 0.490 by 0.399 mm. On one plate there are 103 goblets measuring 24 to 52 μ in diameter. The plates agree fairly well with those of 9992, but as seen from the measurements they are somewhat larger. In absence of male specimens the specific determination is at present provisional, but I believe the specimens are specifically identical with 9992.

B. A. I. 4363, collected by W. B. Lincoln, January 18, 1906, at Bellaire, from *Equus caballus* (the host came from Oklahoma), agrees fairly will with Banks's specimen of *D. nigrolineatus.* A stigmal plate of a (nymp?) of 4363 is shown in figure 25.

Although the material at hand is not in very good condition, the characters given in the specific diagnosis could be recognized.

Salmon's Dermacentor—DERMACENTOR SALMONI new species.

(Figs. 26–36, 65, 66, 79, 95, 96, 114, 115, 131, 132.)

1901: *Dermacentor reticulatus* (Fabricius) of Salmon and Stiles, 1901a, 448–452, figs. 61, 169–177 [North American specimens only].—Banks, 1907, 608 (as syn. of *D. albipictus*); 1908, 55.—Hunter and Hooker, 1907, November 2, 50.

1910: *D. salmoni* Stiles, 1910, 55, figs. 26–36a, 65, 66, 79, 95, 96, 114, 115, 131, 132 (type host *Bos taurus*, in Oklahoma; type U. S. P. H. & M. H. S. 3191).

Specific diagnosis.—*Dermacentor* (p. 18): ♂, young and replete ♀: Caudal margin very bluntly rounded, anus nearer caudal than lateral margin. Color exceedingly variable, very light to very dark [when alive, No. 3179 changed color while being drawn, see figs. 169–172 of Salmon and Stiles, 1901a]. Dorsum: Eyes rather distinct. Scutum well provided with rust; punctations large and small. Venter: Genital pore surrounded by numerous hairs, especially in male. Anal ring nearly circular; in specimens mounted under pressure, 224 to 368 μ in longitudinal by 288 to 385 μ in transverse diameter. Stigmal plates with or without dorso-lateral prolongations; aperture (macula) rather elongate, not strikingly large; goblets about 50 to 100 in number, 26 to 85 μ in diameter, closely set and occupy nearly entire surface [these goblets correspond to the large wart-like punctations shown in figs. 176–177 of Salmon and Stiles, 1901a]; slightly deeper focus shows a finer structure of somewhat reticulate appearance, represented by the middle layer [and corresponding to the finer punctations in figs. 176–177 of Salmon and Stiles, 1901a]; in some parts of the plate, especially near the margin, these fine circles are visible at the same focus which shows the goblets, especially when the plate is slightly curved; the meshes vary in size and shape but may attain 8.8 to 17.6 μ in diameter; the deeper pores (stems of goblets) are about 8.8 μ in diameter; as focus is raised, these circular canals assume a compressed, slit-like outline. Capitulum: Postero-lateral projections short. External article apparently with either two or three teeth, the terminal tooth exceedingly minute, and apparently sometimes lacking, at least not visible, the subterminal tooth larger, proximal tooth largest. Hypostome, each half with small subterminal denticles, followed by three rows of strong denticles, followed proximally by smaller scale-like denticles. Palpi, article 1 triangular to nearly quadrangular ventrally, with two to four ventro-median marginal bristles; article 2 with four to eight such bristles; article 3 slightly broader than long, its apex very blunt so that the article may appear almost quadrangular; a few subterminal and terminal bristles present; dorso-retrograde prolongation indicated on postero-median margin. Legs: The lateral spur on coxæ I may be slightly longer than the median spur; lateral spur of coxæ II–IV unusually sharp; trochanter I with dorso-distal rather prominent retrograde spur; terminal spur on tarsi I–IV usually well developed.

Male.—Length 4.1 to 5.7 mm. long (exclusive of hind legs); greatest breadth 2.3 to 3.6 mm., near stigmal plate. Body rather oval, sides (unmounted) decidedly convex; 0.96 to 1.12 mm. broad at scapulæ. Dorsum: Scutum covers entire dorsum (except capitulum); scapulæ project 0.16 to 0.32 mm. from border of excavation; cervical and marginal grooves distinct; pseudoscutum not at all prominent; scutum deep reddish brown, usually concealed for the most part by a silver white metallic rust with rose and green tinges; when rust is pronounced, there remain a number of reddish to brown spots more or less symmetrically placed; the most prominent are: (a) Four elongate spots arranged in a semicircle or nearly so and running longitudinally; (b') two elongate lateral spots posterior of these, one on each side; (b^2) a dark median spot between, on same plane as b' and covering foveolæ; (c) an elongate median spot and two elongate submedian spots; (d) the forked spot may be very prominent; several dark spots may be present in the postero-lateral festoons; numerous punctations present, with hairs. Marginal groove rather distinct. Foveolæ about at equator, and measuring 64 to 112 μ in diameter. Venter: Ventral surface with hairs and punctations which are noticeably more numerous around genital pore. Genital pore between coxæ II. Stigmal plates 0.518 to 0.714 mm. long by 0.294 to 0.406 mm. broad; goblets may attain 61 in number and 26 to 73 μ in diameter. Capitulum: Length, 0.480 to 0.640 mm. dorsally, 0.80 to 0.896 mm. ventrally; base, 0.48 to 0.576 mm. broad, only about half as long; postero-lateral projections may attain 112 μ long. Mandibles: 0.896 to 1.056 mm. long; digit, 120 μ; dorsal process of internal article elongated transversely, with two well-developed visor-like ridges. Hypostome with numerous subterminal minute denticles which extend an unusual distance proximally in median line; about seven to eight large denticles in each row, followed by scale-like denticles. Each palpus 400 to 576 μ long, 224 to 320 μ broad; article 1 nearly quadrangular, with two to four ventro-median bristles; article 2 with five to six, article 3 with three to four bristles on ventro-median margin. Legs: Coxæ IV large, of two types, the caudal margin may extend to plane of caudal margin of stigmal plate.

Female.—Dorsum: Scutum 1.32 to 2.03 mm. long in median line; 1.376 to 1.84 mm. broad at lateral angles; decidedly emarginate anteriorly; scapulæ project about 0.24 to 0.352 mm. from border of emargination; breadth of scutum at shoulders (unmounted) 1.0 to 1.28 mm.; from shoulders, the sides of the scutum diverge in a somewhat irregularly curved or in a nearly straight line to the lateral angles, then converge in a straight or irregular line to the blunt postero-lateral angles, when they change direction, converging more rapidly to the bluntly rounded posterior angle; the postero-lateral angle may be effaced. Cervical grooves may be rather pronounced, diverging anteriorly and posteriorly from a transverse plane passing through the eyes; they are rather deep anteriorly, shallow and broad posteriorly; they divide scutum into three distinct longitudinal areas; surface of scutum provided with a number of more or less uniformly distributed fine punctations, each bearing a short bristle or hair; on slightly deeper focus they show also a number of larger structures composed of more or less circular spots, apparently forming the insertion of muscle fibers. Eyes light or dark, rather prominent, at lateral angles; rust well distributed, but absent at lateral angles and in grooves; rust may also be absent from a portion of median line and from a divergent line laterally of each posterior branch of cervical groove. Uncovered portion of dorsum shows coarser and finer punctations, with hairs, a distinct median and two submedian furrows; short furrows may appear antero-laterally of submedian furrows; marginal furrow not distinct. Foveolæ round to oval, 64 to 144 μ in diameter. Venter: Punctations and fine hairs present, genital grooves run convex (laterad) caudally to a point slightly caudad of plane of coxæ IV, then they diverge toward the lateral margin; median postanal groove distinct in unmounted specimens. Stigmal plates 0.539 to 0.840 mm. long; 0.416 to 0.602 mm. broad; dorso-lateral projections short or absent; goblets attain about 70 in number and 26 to 85 μ in diameter. Capitulum: 0.608 to 0.768 mm. long (dorsally); base 0.608 to 0.704 mm. broad; 230 to 320 μ long from posterior margin to plane over palpi; dorso-lateral angles not very prominent, project 48 to 80 μ, blunt; porose areas deep, distinct and round, but in one specimen they meet in median line. Mandibles attain 0.8 to 1.13 mm. in length, digit 128 to 144 μ; dorsal process apparently bidentate, with base near terminal extremity. Smaller anterior denticles of hypostome confined to smaller area than in male, may be nearly absent; seven to ten large denticles in each longitudinal row, continued proximally by scales; first transverse row of large denticles may be somewhat irregular and may show four teeth. Palpi 0.448 to 0.608 mm. long by 0.240 to 0.324 mm. broad; dorsal retrograde projections small; article 2 about as broad as long; article 3 broader than long; its apex blunt so that it appears almost quadrangular dorsally; article 1 triangular ventrally, with three to four bristles; article 2 with four to eight bristles; article 3 with two to three bristles. Legs: Coxæ diverge I to IV; lateral spine of coxæ I may attain 256 μ long; ventral margin of protarsus and tarsus II, femur, tibia, protarsus and tarsus III, and tibia, protarsus, and tarsus IV may have small knobs.

Replete female.—May attain 17 mm. long by 12 mm. broad. Form varies with degree of repletion; legs I are lateral of scutum; from here body increases rapidly in breadth, reaching a maximum at about coxæ IV; there may be a constriction at plane of the feveolæ or at stigmata; posterior margin is very bluntly rounded, so that anus may be nearer posterior than lateral margin. (See Salmon and Stiles, 1901a, figs. 171-172). Foveolæ slightly preequatorial. Color is exceedingly variable; live specimens may change color while under observation, especially if background is changed; live specimens may be whitish to creamy color to a greenish or brown; alcohol specimens may be greenish to brown or even black. Dorsum: Dorsal surface may show two prominent longitudinal grooves, diverging slightly caudad and interrupted just caudad of foveolæ; a median groove extending from near foveolæ about to anus. Venter: Vulva may shift slightly forward of coxæ II or even to plane of spines on coxæ I; genital and anal grooves prominent. Legs: Coxæ I–IV diverge markedly.

Nymph.—A nymphal skin, about 4.5 mm. long, shows a stigmal plate 192 by 152 μ, with 17 goblets.

Type.—B. A. I. 3179.

Hosts.—Horse (*Equus caballus*) in Tennessee; cattle (*Bos taurus*) in Oklahoma; collected also in Montana, and (?) Oregon.

Salmon and Stiles (1901a, 448–452, figs. 61, 169–177) confused with *Dermacentor reticulatus* of Europe certain American ticks which prove, from a study of the stigmal plates, to be distinct from the European form.

B. A. I. 2998 (see fig. 176 of Salmon and Stiles, 1901a) consists of five mounted specimens, one male, four females, none in very good condition. They were taken from *Bos taurus;* locality unknown; determined by Banks in 1898 as *D. 5-striatus;* determined by Stiles in 1901 as *D. reticulatus*. The stigmal plates are quite distinct from those of *D. variegatus* and of *D. nigrolineatus*. They present the following measurements in mm.:

Stigmal plates.	Body.	Size of scutum.	Breadth of base of capitulum.	Remarks.
Male: 0.714 × 0.490				
Female: 0.658 × 0.420; 0.616 × 0.420	ca. 12 × 10	(?)	0.576	Mounted, pressed.
Female: 0.658 × 0.504	ca. 10 × 8	1.68 long.	.784	Do.
Female: 0.623 × 0.448	6.6 × 4.86	1.456 × 1.376	.640	Do.
Female: 0.539 × ——; 0.574 × 0.364	ca. 11 × 7	1.328 × 1.42	.688	Do.

There is a very slight tendency, both in the male and in the female, to the formation of a dorso-lateral projection. The goblets (fig. 27) may attain 79 in number and 32 to 80 μ in diameter. The trochanter has a dorso-distal retrograde spur.

U. S. P. H. & M. H. S. 9724 contains two specimens (one male, one female), collected in Montana by Maj. P. M. Ashburn, U. S. Army; host is not given (horse ?); they possess certain striking resemblances to 2998, yet certain differences are noticed. Whether these differences are of specific value or individual variations can not be determined at present because of paucity of material. The following are the measurements in mm.:

Stigmal plates.	Body.	Size of scutum.	Breadth of base of capitulum.	Remarks.
Male: 0.518 × 0.378; 0.518 × 0.378	4.129 × 2.331		0.480	Mounted, pressed.
Female: 0.644 × 0.476; 0.644 × 0.476	4.928 × 2.797	1.84 × 1.84	.688	Do.

The striking difference to be noticed is the regularity of outline of the male plate as compared with that of 2998; the female plate agrees more closely with that of 2998. In number the goblets are 54 to 58 in the male and 51 to 55 in the female and 30 to 85 μ in size. Figures 28-31 show the two pairs of stigmal plates from two specimens and indicate that we have here a species which may exhibit considerable variation.

B. A. I. 3179 (compare figs. 169-172 of Salmon and Stiles, 1901a) contains four mounted specimens of fragments of three females; they were collected from *Bos taurus* in Oklahoma by W. F. Cantalow; determined by Stiles in 1901 as *D. reticulatus*. They appear to agree with 2998. The measurements, in millimeters, are as follows:

Stigmal plates.	Body.	Size of scutum.	Breadth of capitulum	Remarks.
Female: 0.672 × 0.560; 0.686 × 0.560		2.03×1.76	0.752	Mounted, pressed.
Female: 0.616×0.518; 0.630×0.518	ca. 6×5			Do.
Female: ?0.840 × ——; ?0.800 × 0.608	ca. 8×6			Do.

This material is not very satisfactory, but, despite the large stigmal plate in one specimen, it is impossible at present for me to distinguish between these ticks and 2998. Figure 26, from this material, shows 78 goblets, 26 to 65 μ in diameter.

B. A. I. 3206 contains two mounted and twelve unmounted females, collected by Doctor Steddom from *Equus caballus* at Nashville, Tenn., February 27, 1901. Here again the general agreement of the plates with 2998 is very striking, despite certain pronounced differences. Still, as the plates on the two sides on single mounted specimens vary one from the other, it does not seem altogether justified to consider the form distinct from 2998. The millimeter measurements of the mounted specimens are as follows:

Stigmal plates.	Body.	Length of scutum.	Breadth of capitulum.	Remarks.
Female: 0.616×0.504; 0.616× ——	ca. 14×12			Mounted, pressed.
Female: 0.630×0.462				Do.

The goblets are about 71 in number and vary from 28 to 48 μ in diameter. A gravid, unmounted female attains about 17 mm. in length by 12 mm. in breadth.

U. S. P. H. & M. H. S. 10003, collected by Dr. E. C. Stevenson, contains four males and one female presenting the following measurements in mm.:

Stigmal plates.	Body.	Size of scutum.	Breadth of capitulum.	Remarks.
Male: 0.588×0.336	5.43×3.19		0.576	Mounted, pressed.
Male: 0.602×0.322; 0.602×0.280	5.46×3.26		.576	Do.
Male: 0.638×0.364; 0.700×0.294	5.72×3.66		.640	Do.
Male: 0.700×0.350; 0.672×0.406	5.66×2.79		.608	Do.
Female: 0.560×0.420; 0.560×0.420	5.06×3.19	1.696×1.680	.768	Do.

These specimens present very marked variations among themselves and when compared with the other specimens listed here. The female plate agrees fairly well with 2998, but the male plates are quite different, being of a much more elongate pattern. Figure 36 shows a plate with 61 goblets measuring 30 to 60 μ in diameter. The variation in form and size of coxæ IV of the males is very striking.

The following specimens have been examined:

B. A. I. 2998. Host *Bos taurus;* loc. ? ; 1898; determined by Banks, 1898, as *Dermacentor 5-striatus;* determined by Stiles, 1901, as *D. reticulatus.*

B. A. I. 3179. Host *Bos taurus;* Oklahoma; collected by W. F. Cantalow; determined by Stiles, 1901, as *D. reticulatus.*

B. A. I. 3026. Host *Equus caballus;* Nashville, Tenn.; collected by Steddom, February 27, 1901.

U. S. P. H. & M. H. S. 9724. Host— ? ——— ; Montana ; collected by Maj. P. M. Ashburn, U. S. Army.

U. S. P. H. & M. H. S. 9756. Male and female from U. S. National Museum ; bears the label "*Ixodes oregonensis*," but without further data.

U. S. P. H. & M. H. S. 9769. Belongs to U. S. National Museum, original No. 1348 ; no further data.

U. S. P. H. & M. H. S. 10003. Received from Dr. E. C. Stevenson.

In the specimens just listed, we evidently have *dermacentors* which agree very closely in respect to their goblets, scuta, and base of capitulum. The outline of the stigmal plate varies exceedingly, its size moderately. In order to determine whether these variations are individual or specific, it is desirable to have a large number of specimens taken from one host animal. At present, despite the variations mentioned, I do not feel justified in separating the forms into distinct species, although it seems very possible that 10003 may eventually be separated out as a distinct systematic unit.

The original determination of *D. reticulatus* for this species was based upon specimens found in the collection of the Bureau of Animal Industry. Later, they were recognized as distinct. Banks (1907, 608), on basis of the figure Salmon and Stiles (1901a, fig. 177) published of the stigmal plate identified the form with *D. variegatus*= *D. albipictus*, but in this he has been led into error.

DERMACENTOR ALBIPICTUS Packard, 1869 seu VARIEGATUS Marx, 1897.

(Figs. 37–39, 67, 68, 80, 97, 98, 116, 117, 133.)

1868 : *The Moose Tick* Hays and Packard, 1868, December, 559, fig. 1a–e (on the Moose ; Nova Scotia).

1869 : *Dermacentor albipictus* Packard, 1869, 662–663 ; 1869, 365–366.

1897 : *D. variegatus* Marx and Neumann, in Neumann, 1897a, 367–370, 383, figs. 22–24 (type locality U. S. A.).—Salmon and Stiles, 1901a, 452–454, figs. 178–185.

Banks (1907, 608) has identified *D. variegatus* with *D. albipictus*. He tells me that he has examined the type of both and that there is no question regarding their identity. On his authority they are here accepted as synonymous. He also considers "*D. reticulatus*" of Salmon and Stiles as belonging to the same species, but as will be shown in this paper I can not concur with him in this view.

The specimens used as basis for the present discussion of *D. albipictus* are *D. variegatus*.

SPECIFIC DIAGNOSIS.—*Dermacentor* (p. 18) : ♂ and young ♀ : Caudal margin very blunt, anus being nearer posterior than lateral margin. Color red to deep reddish brown, except for rust ; capitulum and legs lighter. Dorsum : Eyes not very prominent. Scutum prominently emarginate cephalad ; rather well provided with whitish rust, which varies considerably in different specimens ; punctations and hairs numerous. Venter : Genital pore surrounded by numerous hairs, especially in male. Anal ring nearly circular, 320 μ long by 368 μ broad. Stigmal plates may be about as large as female coxæ IV ; with or without short dorso-lateral prolongation ; aperture rather prominent ; goblets large, rather irregular in outline, 108 to 126 in number, 8 to 59 μ in diameter, closely set and occupy nearly or quite entire surface (see also Salmon and Stiles, 1901a, figs. 184, 185); meshes of middle layer about 8 μ in diameter; stem of goblets about 8 μ in diameter, circular on lower focus, but compressed, slit-like on higher focus. Capitulum: Postero-lateral projections of base 80 to 124 μ long, blunt. External article with three teeth, the proximal tooth large, next distal tooth smaller, disal tooth apical and often scarcely visible. Hypostome, each half with an unusually large number of small subterminal denticles, followed by three rows of strong denticles, followed proximally by small scale-like denticles. Palpi, article 1 nearly quadrangular ventrally, provided with four to five ventro-marginal bristles; article 3 slightly broader than long, its apex somewhat rounded, a few dorso-terminal and terminal bristles; dorso-retrograde prolongations of article 2 indicated on postero-median margin. Legs: The lateral spur of coxæ I slightly longer than the median; lateral spurs of coxæ II–IV unusually sharp; trochanter I with a dorso-distal rather prominent retrograde spine; terminal recurved spur on tarsi I–IV well developed.

Male.—Length 6.5 mm. (exclusive of hind legs; with hind legs outstretched, about 8 mm.); greatest breadth 4.3 mm. Body subtriangular; unmounted specimens 1.12 mm. broad at scapulæ, specimens mounted under pressure may measure up to 1.2 mm. at this point; the sides diverge from here in nearly a straight or in a curved line until they reach their maximum divergence about at stigmal plate. Dorsum: Scutum covers entire dorsum (except capitulum); scapulæ project 0.40 to 0.48 mm. from border of excavation; cervical groove very deep anteriorly, marginal groove distinct; scutum deep reddish brown, well provided with rust; pseudoscutum usually quite prominent, extending about 0.35 of length of scutum in median line; the most prominent red spots back of pseudoscutum are: (a) Four elongate spots arranged radially or nearly so from caudal margin of pseudoscutum; (b) two elongate spots back of these, one on each side of the scutum; (c) an elongate median and two elongate submedian spots; (d) forked spot very prominent; spots also present on postero-lateral margins; numerous minute punctations and some slightly larger punctations, with hairs present. Foveolæ about in equator of scutum and measuring 96 μ in diameter. Venter: Ventral surface finely punctate with numerous minute white hairs, noticeably more numerous and larger punctations around genital pore. Genital pore between coxæ II. Stigmal plates 0.688 to 0.848 mm. long (through the aperture); breadth of plate (including prolongation when present) 0.48 to 0.683 mm.; goblets attain 126 in number and 15 to 59 μ in diameter. Capitulum: 0.700 to 0.816 mm.; base 0.688 to 0.80 mm. broad, and 0.368 mm. long from posterior margin to anterior margin over palpi; postero-lateral projections rounded, 128 μ long. Mandibles 1.1 to 1.2 mm. long; digit 185 μ; dorsal process of internal article elongate transversely in two nearly parallel ridges like two visors of a cap. Hypostome with six to eight large denticles in each row. Palpi 0.56 to 0.672 mm. long, each palpus 0.32 to 0.352 mm. in maximum breadth; shorter than in female, especially article 2; article 2 with six to seven ventro-median bristles; article 3 with three to four ventro-median bristles. Legs: Coxæ IV large, the caudal margin attaining plane of caudal margin of stigmal plate, the median margins nearly parallel for some distance; ventral spurs or knobs may be present on tarsus I, protarsus and tibia II, and especially on femur, tibia, protarsus, and tarsus IV; tarsi II to IV with subterminal spur.

Female.—Dorsum: Scutum 1.776 to 2.128 mm. long in median line; 1.8 to 1.9 mm. broad at lateral angles; very prominent because of size and rust; scapulæ project about 0.468 mm. from border of excavation; from shoulders sides of scutum diverge in rather strongly convex line to lateral angles, then converge in straight or somewhat convex line to postero-lateral angles, then they converge more rapidly to the bluntly rounded posterior angle. Cervical grooves diverge anteriorly and posteriorly from plane passing through the eyes, deeper anteriorly, not especially broad posteriorly; eyes at lateral angles; punctations fine and numerous; color light brown with thin white rust of slightly metallic effect and leaving exposed a median longitudinal band, the cervical grooves, a small band laterally of posterior branch of grooves, and a distinct brown spot median of eyes. Uncovered portion of dorsum shows numerous punctations with hairs, a distinct median, two submedian, and well marked marginal grooves; additional grooves may appear between submedian and marginal grooves. Foveolæ round to oval, 128 to 144 μ in diameter, a short distance caudad of scutum. Venter: Numerous punctations and fine hairs present; genital grooves run subparallel to a point about midway between coxæ IV and anus, whence they diverge markedly toward lateral margin. Stigmal plates 0.736 to 0.784 mm. long by 0.64 to 0.70 mm. broad; goblets 8 to 49 μ in diameter, may attain 108 (in nymph 14) in number. Capitulum: 0.8 to 1 mm. long; base 0.768 to 0.8 mm. broad; 0.288 mm. long from posterior margin to anterior margin over palpi; postero-lateral prolongations 80 μ; porose areas deep, roundish to rather oval, diverging anteriorly. Mandibles attain 1.28 to 1.42 mm. long; digit 180 μ long; internal apophysis (dorsal process) distinctly tridentate, the median tooth shortest. Smaller anterior denticles of hypostome numerous; nine to ten large denticles in each row. Palpi may attain 0.768 mm. ventrally, by 256 to 320 μ broad, not extending laterally of base of capitulum; article 2 longer than broad; dorso-retrograde projection small; article 3 slightly broader than long, its apex rounded; article 1 with seven ventro-median bristles; article 2 and with nine ventro-median bristles; article 3 with three ventro-median bristles. Legs: Coxæ diverge II to IV; lateral spine of coxæ IV may attain 320 μ long; ventral margin of tibia and tarsus II, femur, tibia, protarsus and tarsus III, and tarsus IV may have small knobs.

Young female.—Length 5.5 to 6 mm. (exclusive of hind legs; with hind legs extended may attain 8 mm.); greatest breadth 3 mm. Body rather subtriangular, depressed, sides may be nearly straight and nearly parallel for some distance; unmounted specimens 1.28 mm. broad at scapulæ, specimens mounted under pressure may attain 1.28 mm. at this point; the sides diverge from here to the maximum breadth about at coxæ IV or at stigmal plate. Dorsum: Scutum covers about 0.40 of length of body (exclusive of head); eyes distinct but not prominent. Venter: Vulva between coxæ II.

Replete female.—May attain 17 mm. long by 10 mm. broad. Form varies with degree of repletion.

Nymph.—Length 5 to 6 mm.; breadth 3 to 3.5 mm.; sides nearly parallel; both ends bluntly rounded; anus nearer lateral than posterior margin. Goblets about 14 in number.

TYPE.—Type specimen of *D. albipictus* is, teste Banks, in the Museum of Comparative Zoology.

HOSTS.—Moose, wapiti, and beaver.

The stigmal plate in this species was figured by Salmon and Stiles (1901a, figs. 184-185) from specimens not treated with caustic, and the fact was pointed out that it was coarsely punctate. From the new point of view, these coarse punctations represent the goblets.

The first reference to the moose tick appears to be the following:

The moose tick.—On the 13th of April a pair of young moose were brought through New York on their way to Europe. They were raised in Nova Scotia, and being very tame, were allowed to run at large. The cow moose would ramble off in the woods, and while there had become infested with ticks; the bull had escaped contact with these insects. When the cow arrived in New York her sides and back were almost covered with adult ticks. The insects were removed, very much to the relief of the animal. and the ticks were placed in a bottle without food or water. On the 1st of May they commenced to lay eggs, and continued to do so until the 25th of June, when they died. The eggs are forced out in large masses. On the 3d of July, the day after I sent the drawings to you, the entire mass of eggs seemed to hatch out at once, the shell opening like a clam, and releasing a six-legged insect.—W. J. Hays.

[The specimens sent us by Mr. Hays are very interesting, as showing that the young tick has only three pairs of legs instead of four, which all adult spiders and mites (*Arachnida*) possess. This is a strong argument for the supposition that the *Arachnids* form an order in the class of insects and not an independent class. Figure 1 e represents the adult tick, drawn by Mr. Hays. The six-footed young has enormous legs, and the head is separated from the hind body, where in the adult it is sunken in the thorax; d, shows the claws, with a broad sucking disk beneath, enabling it to adhere to objects. On the right is a magnified drawing of the mouth parts of the young; a, is the labium, armed with hooks; b, the maxillæ, probably, also armed with powerful hooks: and c, the mandibles. Thus armed, the young tick buries itself in the flesh of its victim.—Eds.]—Amer. Nat., v. 2 (10), 1868, p. 559.

Packard (1869, September, 365; 1869, 662–663) republished the figures, but added no details except that "the opening of the oviduct is just behind the head, between the anterior pair of feet, so that the eggs appear as if ejected from the mouth."

The specimens taken as basis for the present study are:

B. A. I. 3172. Host Wapiti or moose; Blue Mountain, New Hampshire; determined by Stiles and Hassall, 1899, as *D. variegatus*.

Banks has examined specimens from Adirondack Mountains, New York; Michigan, Nebraska, Montana, Idaho, Nevada, and Washington State.

NITENS GROUP.

DERMACENTOR NITENS Neumann, 1897.

(Figs. 40–43, 69, 70, 81, 99, 100, 118, 119, 134.)

1897: *Dermacentor nitens* Neumann, 1897a, 376–378, fig. 28 (on *Equus caballus;* Jamaica, also Santo Domingo).—Banks, 1908, 50–51, pl. 7, figs. 7, 10; (Jamaica, Santo Domingo, Texas, Haiti).—Salmon and Stiles, 1901a, 455, figs. 215–218 (*Equus caballus;* Porto Rico).

The peculiar structure of the stigmal plates of this species was noticed by Neumann. In the present paper it will be sufficient to show illustrations from the new point of view of the goblets (see figs. 40 to 43).

In a male specimen examined the goblets were five in number and varied from 43 to 88 μ in diameter. In a female, the goblets were six in number and varied from 78 to 115 μ in diameter.

Banks (1908, p. 51) reports this species from Jamaica, Santo Domingo, Haiti, Texas, and Arizona.

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LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar. Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of Trypanosoma lewisi. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis*; by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horse-hair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations on two new parasitic trematode worms: *Homalogaster philippinensis* n. sp., *Agamodistomum nanus* n. sp., by Ch. Wardell Stiles and Joseph Goldberger. 4. A reexamination of the original specimen of *Tænia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

*No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 46—*Hepatozoon perniciosum* (n. g. n. sp.); a hæmogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*). By W. W. Miller.

No. 47.—Studies on Thyroid.—I. The relation of Iodine to the Physiological Activity of Thyroid Preparations. By Reid Hunt and Atherton Seidell.

No. 48.—The Physiological Standardization of Digitalis. By Charles Wallis Edmunds and Worth Hale.

No. 49.—Digest of comments on the United States Pharmacopœia. Eighth decennial revision for the period ending December 31, 1905. By Murray Galt Motter and Martin I. Wilbert.

No. 50.—Further studies upon the phenomenon of anaphylaxis. By M. J. Rosenau and John F. Anderson.

No 51.—Chemical tests for blood. By J. H. Kastle.

No. 52.—Report No. 3 on the origin and prevalence of typhoid fever in the District of Columbia (1908). By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 53.—The influence of certain drugs upon the toxicity of acetanilide and antipyrine. By Worth Hale.

No. 54.—The fixing power of alkaloids on volatile acids and its application to the estimation of alkaloids with the aid of phenolphthalein or by the Volhard method. By Elias Elvove.

No. 55.—Quantitative pharmacological studies—adrenalin and adrenalin-like bodies. By W. H. Schultz.

No. 56.—Milk and its relation to the public health. [Revised edition.] By various authors.

No. 57—I. The presence of tubercle bacilli in the circulating blood in clinical and experimental tuberculosis. By John F. Anderson. II. The viability of the tubercle bacillus. By M. J Rosenau.

No. 58. Digest of comments on the Pharmacopœia of the United States of America (eighth decennial revision) and the National Formulary for the period ending December 31, 1906. By Murray Galt Motter and Martin I. Wilbert.

No. 59.—The oxidases and other oxygen catalysts concerned in biological oxidations. By Joseph Hoeing Kastle.

No. 60.—A study of the anatomy of *Watsonius* (n. g.) *watsoni* of man, and of 19 allied species of mammalian trematode worms of the superfamily *Paramphistomoidea.* By Ch. Wardell Stiles and Joseph Goldberger.

No. 61.—Quantitative pharmacological studies: Relative physiological activity of some commercial solutions of epinephrin. By W. H. Schultz.

No. 62.—The taxonomic value of the microscopic structure of the stigmal plates in the tick genus *Dermacentor.* By Ch. Wardell Stiles.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

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D reticulatus ♂

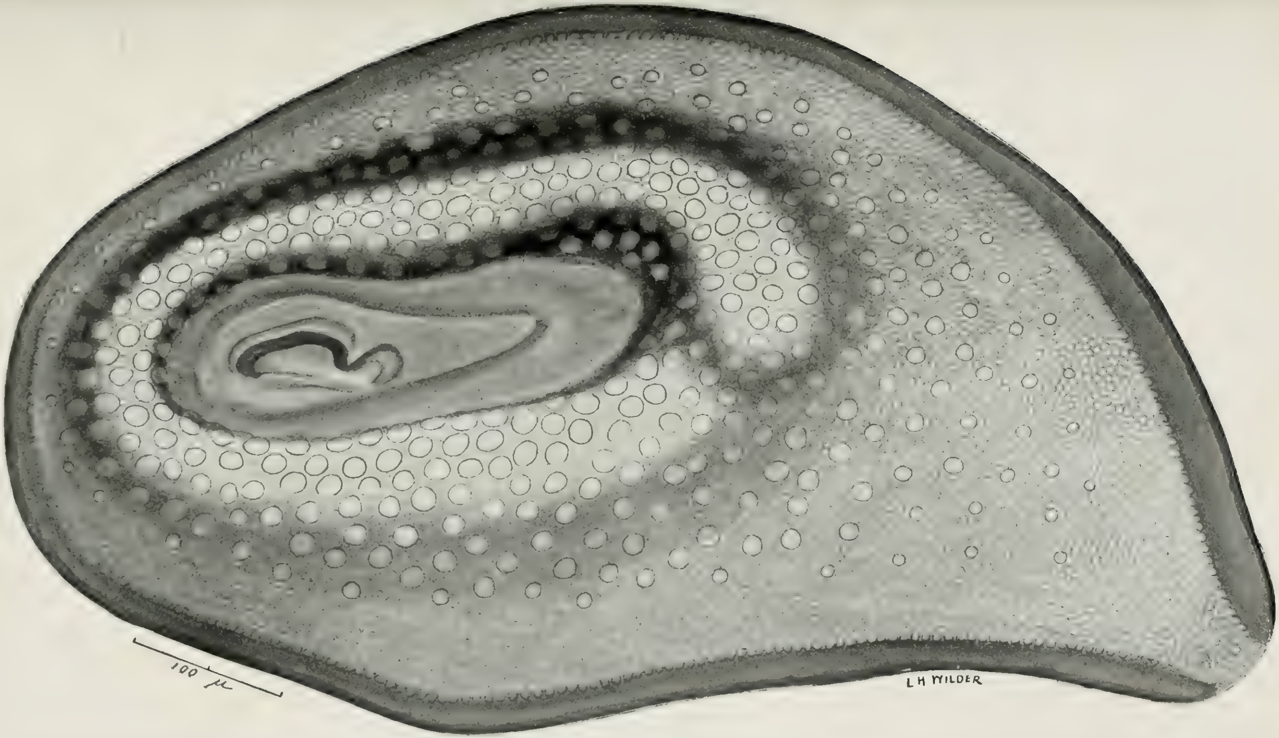


FIG. 1.—Stigmal plate of male of *Dermacentor reticulatus* of France. Notice the elongate form of the plate, with the caudally situated short dorso-lateral prolongation; the aperture is elongate, cephalad, and mediad of the center; the outline of the relatively large chamber under the aperture is seen; the goblets are numerous (378) and are much more thickly set near the aperture than near the periphery; toward the prolongation the goblets become sparse and smaller; the middle layer is visible as composed of very numerous circles which form a mesh work; upon examination with a hand lens the goblets appear as fine punctations, while at the periphery the circles of the middle layer may appear as excessively minute punctations. Greatly enlarged. Original. B. A. I. 3904.

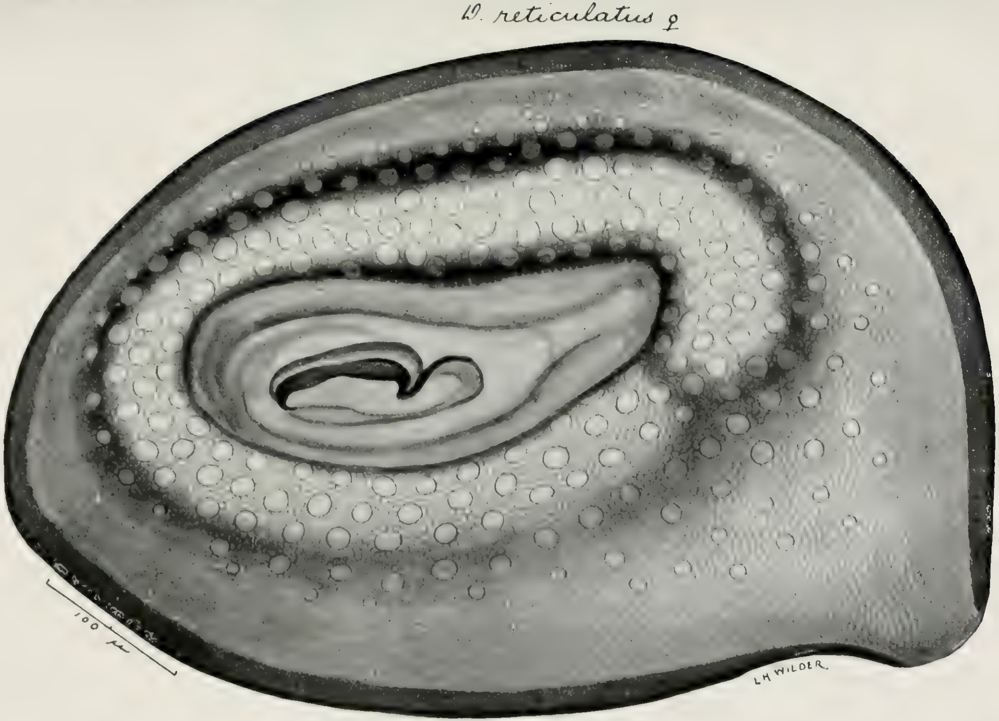


FIG. 2.—Stigmal plate of female of *D. reticulatus*. Notice that this is relatively less elongate than the male plate, but otherwise of the same general structure. Goblets are 215 in number. Greatly enlarged. Original. B. A. I. 3904.

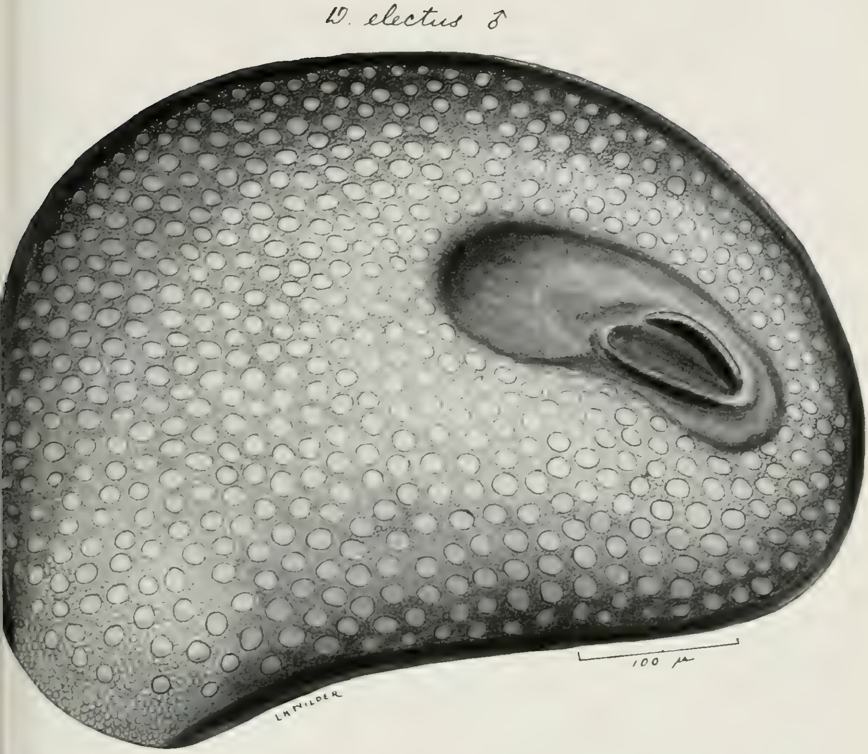


FIG. 3.—Surface view of stigmal plate of male *D. electus* [*D. variabilis*]. Notice the location of the aperture near the ventro-median-anterior margin; the plate is not so elongate as that of *D. reticulatus* and the dorso-lateral prolongation is subterminal; the goblets are 536 in number, rather evenly distributed; the circles of the middle layer are visible. Greatly enlarged. Original. U. S. P. H. & M. H. S. 9920.

D. electus ♂

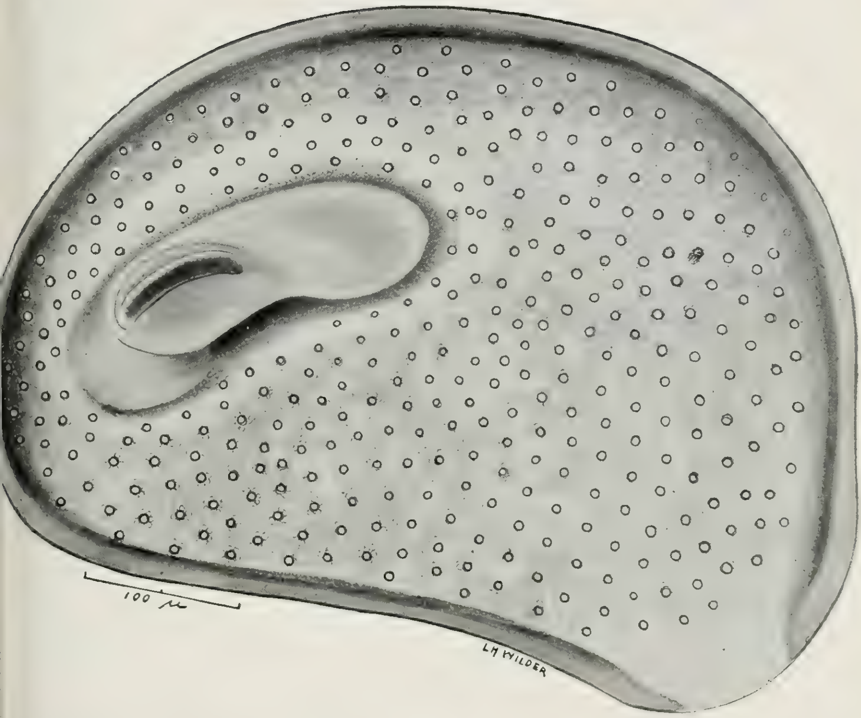
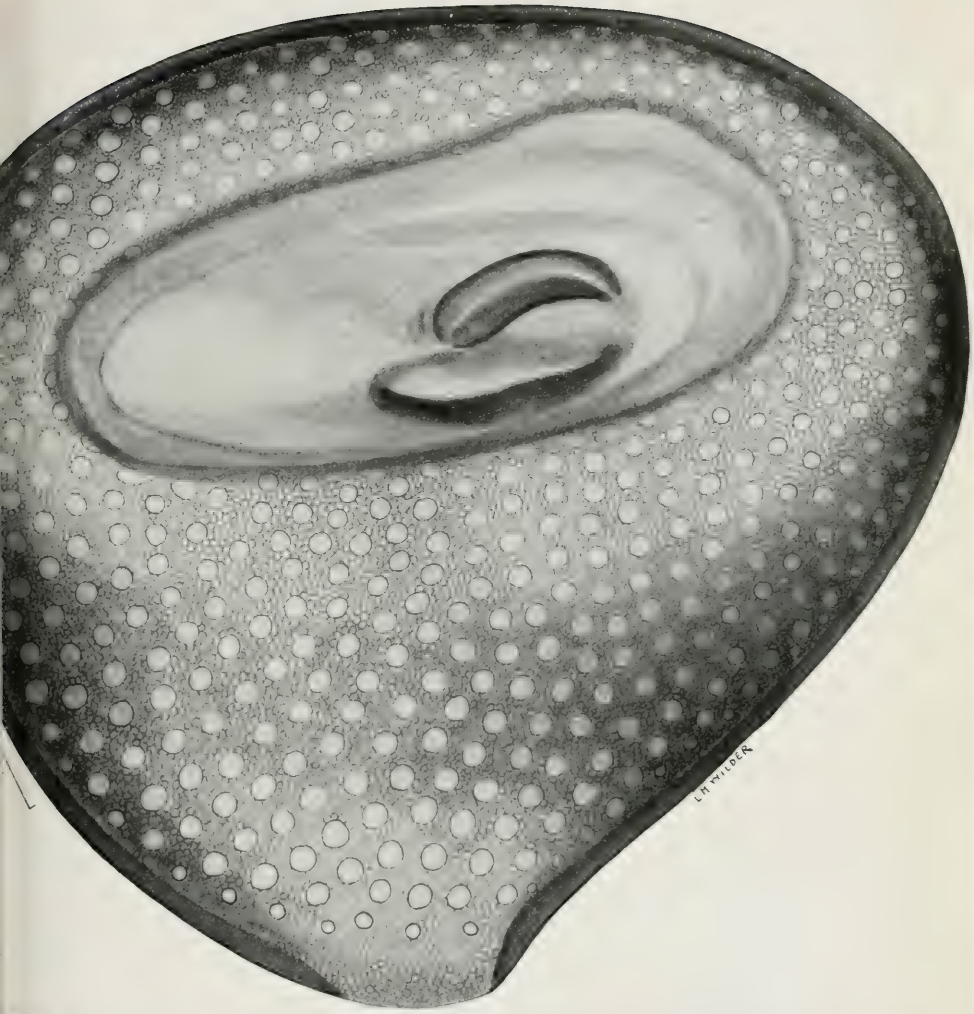


FIG. 4.—Deep focus of plate, showing the relatively small, elongate chamber under the aperture and the numerous circles (goblet stems) of the inner layer. Greatly enlarged. Original. U. S. P. H. & M. H. S. 9920.

D. electus ♀



emale stigmal plate of *D. electus* seu *variabilis*. Notice that this plate is much broader than that of the male; 455 are present, and the chamber is much larger; the dorso-lateral prolongation is just caudad of the equator. enlarged. Original. B. A. I. 3150.

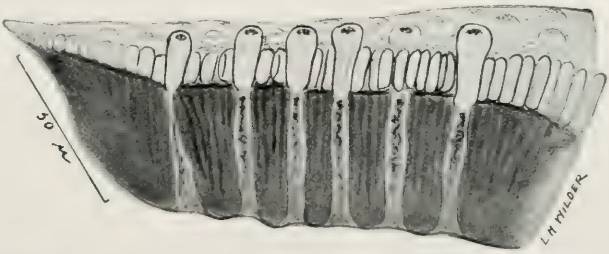


FIG. 6.—Transverse section of a portion of stigmal plate of *D. electus* seu *variabilis*. Notice the three layers: in the upper layer are seen the goblets; in the middle layer are seen the goblets and the chitinous supporting cells; in the thick inner layer are seen the stems of the goblets. Greatly enlarged. Original.

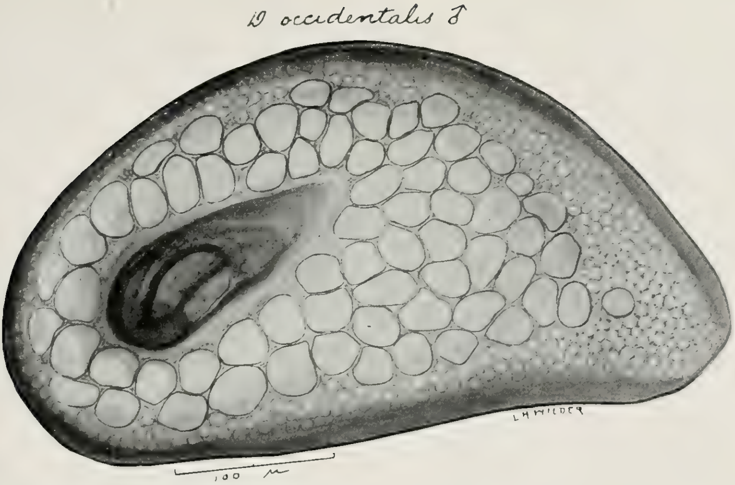


FIG. 7.—Stigmal plate of male *D. occidentalis*. Notice the elongate form, the position of the aperture, and the terminal dorso-lateral prolongation; the goblets (67 in number) occupy a considerable portion of the plate, but are absent from the margin and from the terminal portion of the prolongation; in these places the circles of the middle layer appear. Greatly enlarged. Original. Marx 119, type.

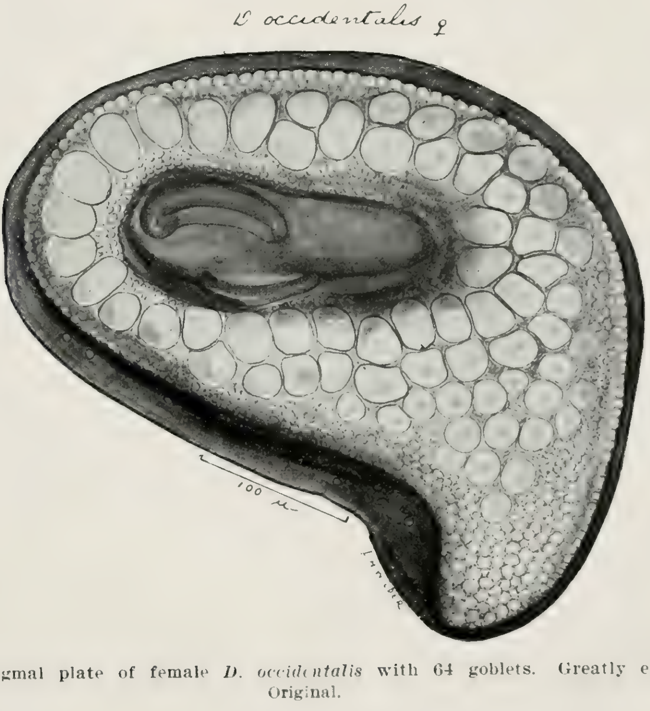


FIG. 8.—Stigmal plate of female *D. occidentalis* with 64 goblets. Greatly enlarged. Original.

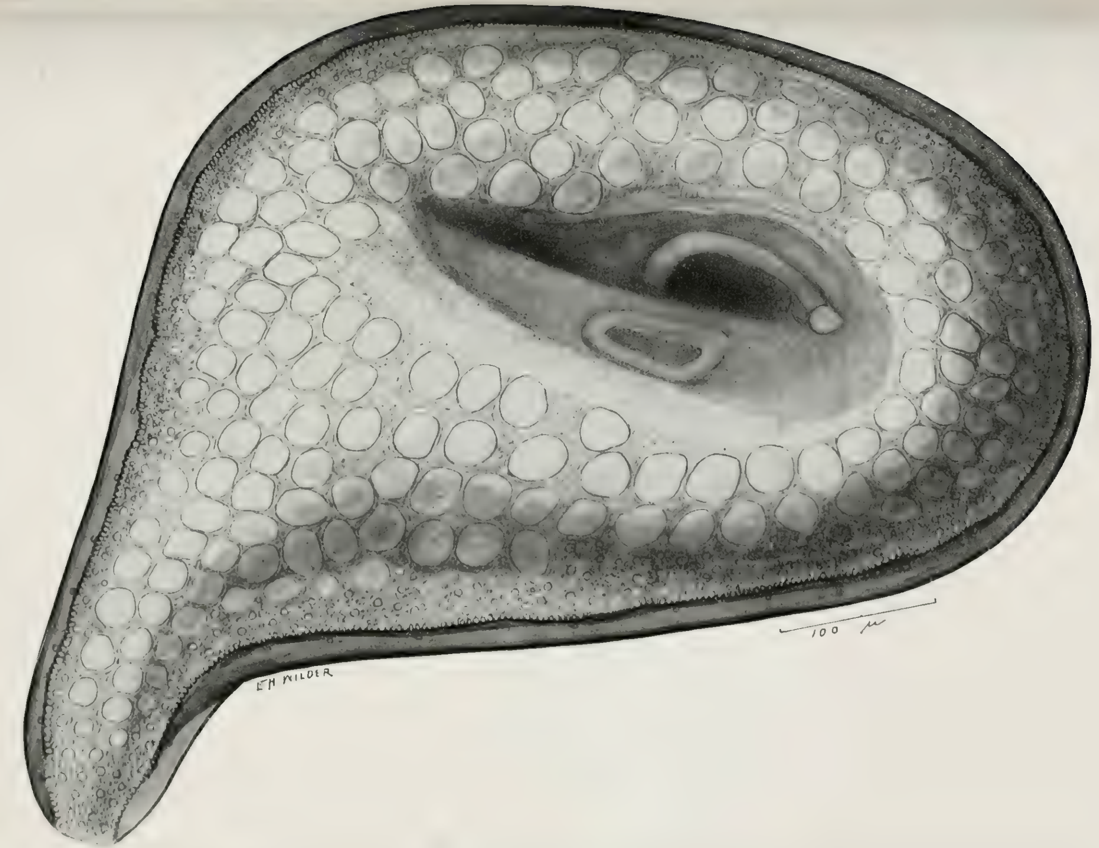


FIG. 9.—Stigmal plate of male *D. andersoni*. Notice the relatively large aperture and chamber and the prominent dorsolateral prolongation which forms a right angle at the caudal margin; the goblets are numerous (157) and evenly distributed, but are absent from the margin; the middle layer is visible. Greatly enlarged. Original. U. S. P. H. & M. H. S. 9156.

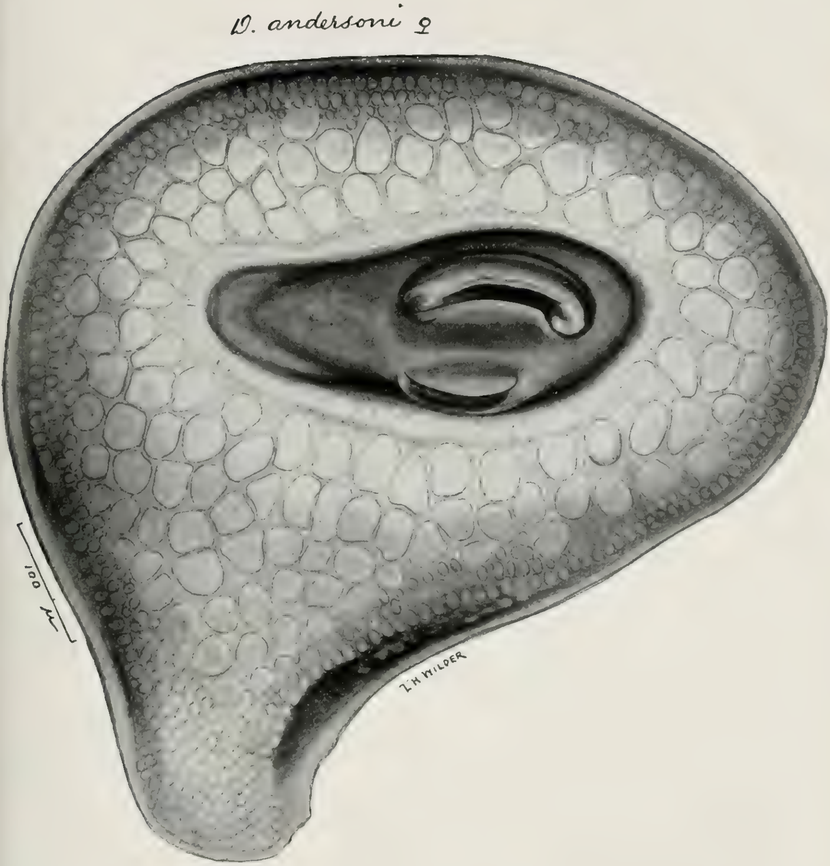


FIG. 10.—Stigmal plate of female *D. andersoni.* Notice the acute angle formed by the dorso-lateral prolongation; the anterior margin of the prolongation is broader than the caudal margin; 120 goblets are present. Greatly enlarged. Original. U. S. P. H. & M. H. S. 9503.

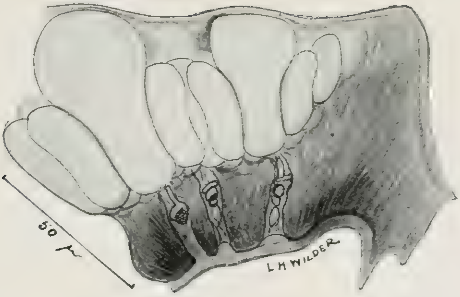


FIG. 11.—Section of a stigmal plate of *D. andersoni*. Greatly enlarged. Original.

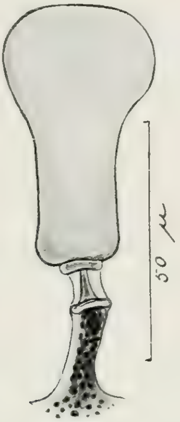


FIG. 12.—An isolated goblet of same. Greatly enlarged. Original.

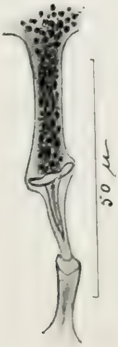


FIG. 13.—An isolated hair near margin of stigmal plate. Greatly enlarged. Original.

D venustus ♂

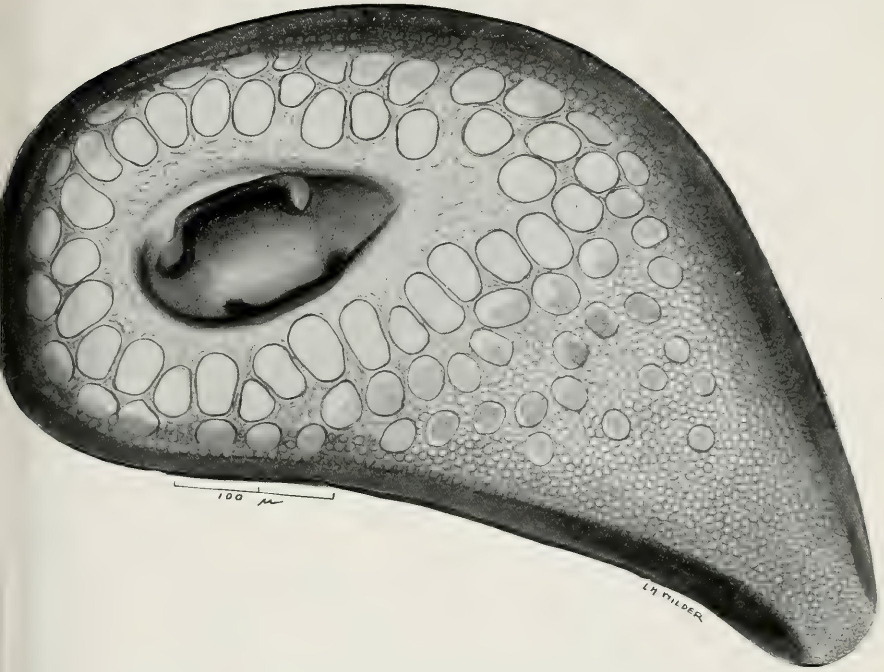


FIG. 14.—Stigmal plate of male *D. venustus*. Notice the broad aperture, the prominent terminal prolongation, and the scattered goblets; the middle layer also is visible. Greatly enlarged. Original. Marx 122.

D. venustus ♀

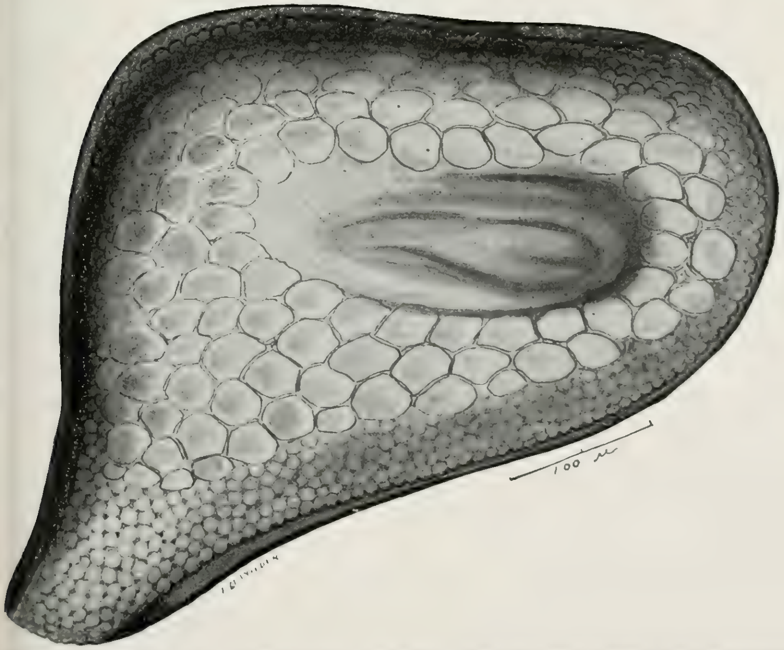


FIG. 15.—Stigmal plate of female *D. venustus*. As this was drawn from an unmounted specimen, it may not be absolutely so exact a representation as is the plate of the male. Greatly enlarged. Original. Marx 122.

D parumapertus ♀

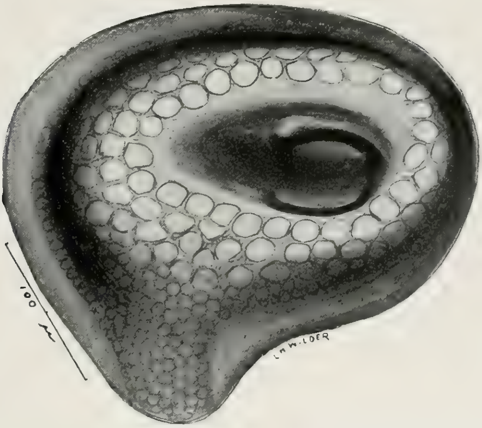


FIG. 16.—Stigmal plate of female *D. parumapertus.* Notice the convexity of the plate, the prolongation which forms an acute angle at the caudal margin of the plate, the concentration of the goblets around the aperture, and note also the unusually large chamber. Greatly enlarged. Original. Marx 143.

D parumapertus marginatus ♂

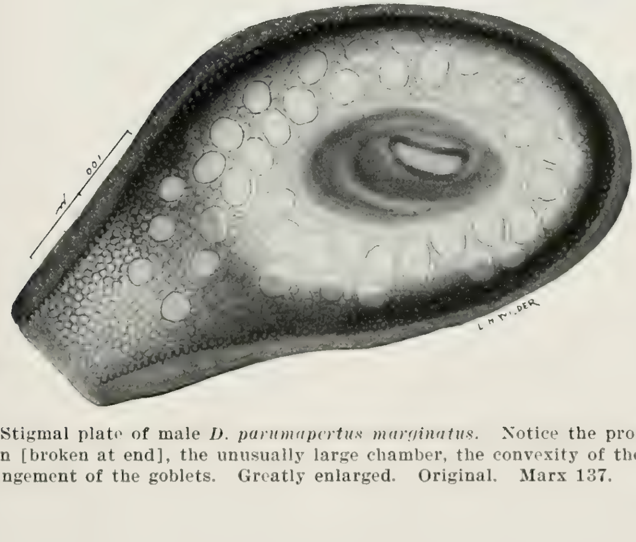
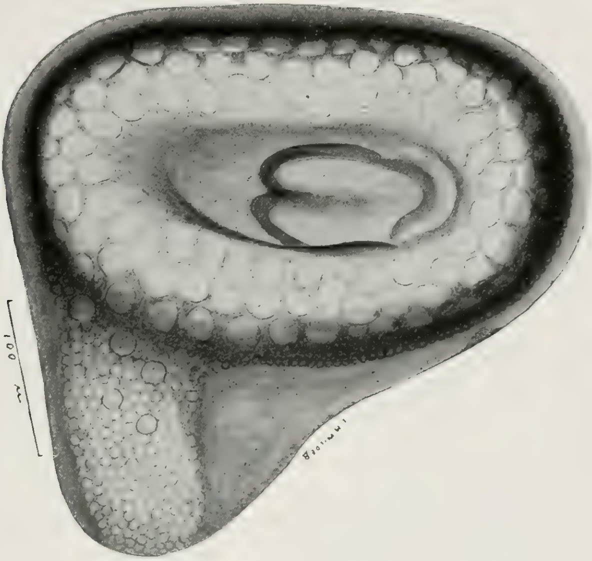
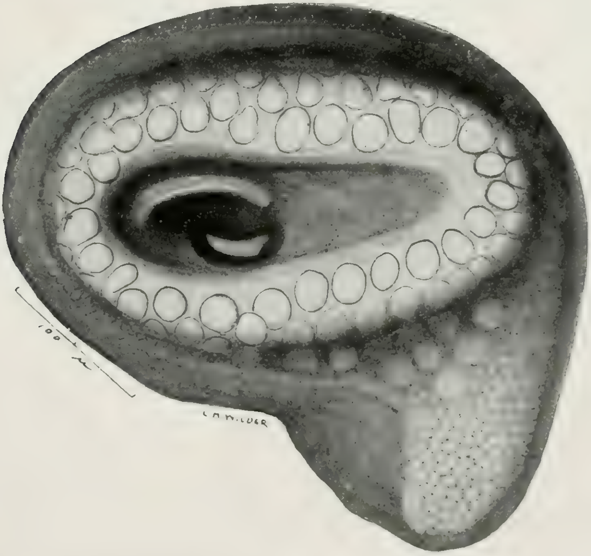


FIG. 17.—Stigmal plate of male *D. parumapertus marginatus.* Notice the prominent prolongation [broken at end], the unusually large chamber, the convexity of the plate, and the arrangement of the goblets. Greatly enlarged. Original. Marx 137.

D. parumapertus marginatus ♀



D parumapertus marginatus ♀



FIGS. 18-19.—Plates of two female *D. parumapertus marginatus*. Notice the acute angle formed by the prolongation, the enormous aperture and chamber, the circular goblets, the convexity of the plate, and the broad anterior margin of the prolongation. Greatly enlarged. Original. Marx 137 and B.A.I.3415.

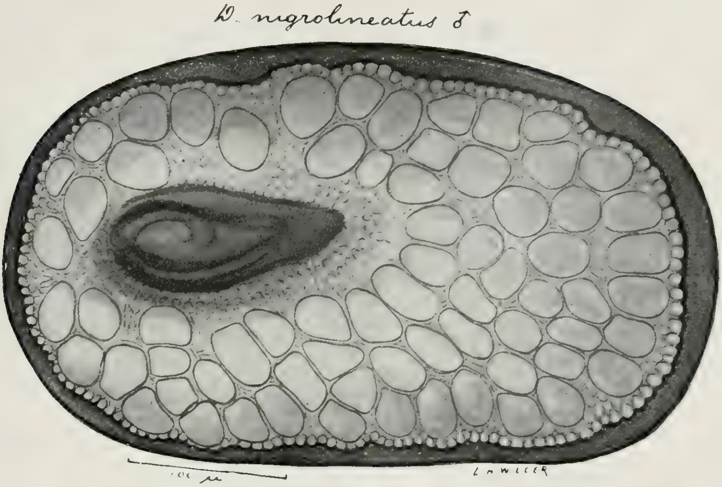


FIG. 20.—Stigmal plate of male tick determined by Banks as *D. nigrolineatus.* Notice the preequatorial position of the aperture, the arrangement of the goblets, and the absence of a dorso-lateral prolongation. Enlarged. Original. U. S. P. H. & M. H. S. 10592.

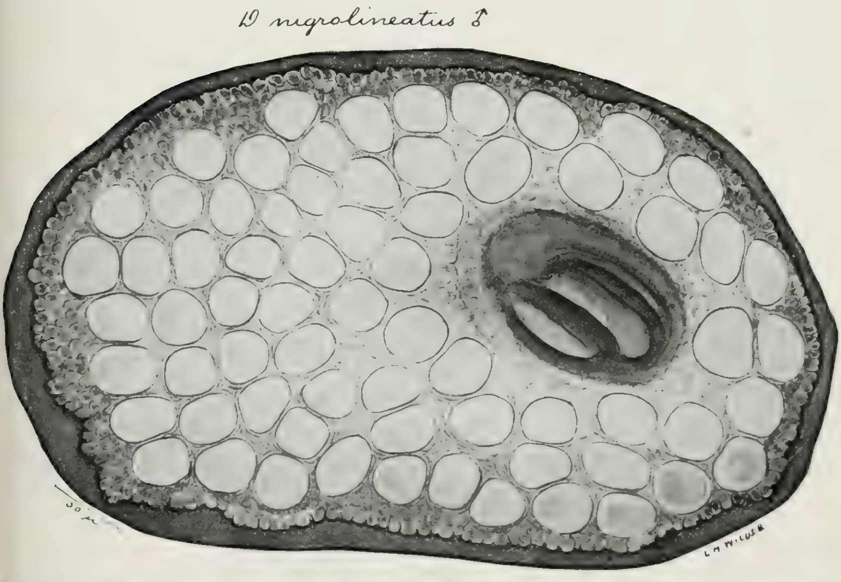


FIG. 21.—Stigmal plate of male *D. nigrolineatus* from Oklahoma. Enlarged. Original. B. A. I. 4363.

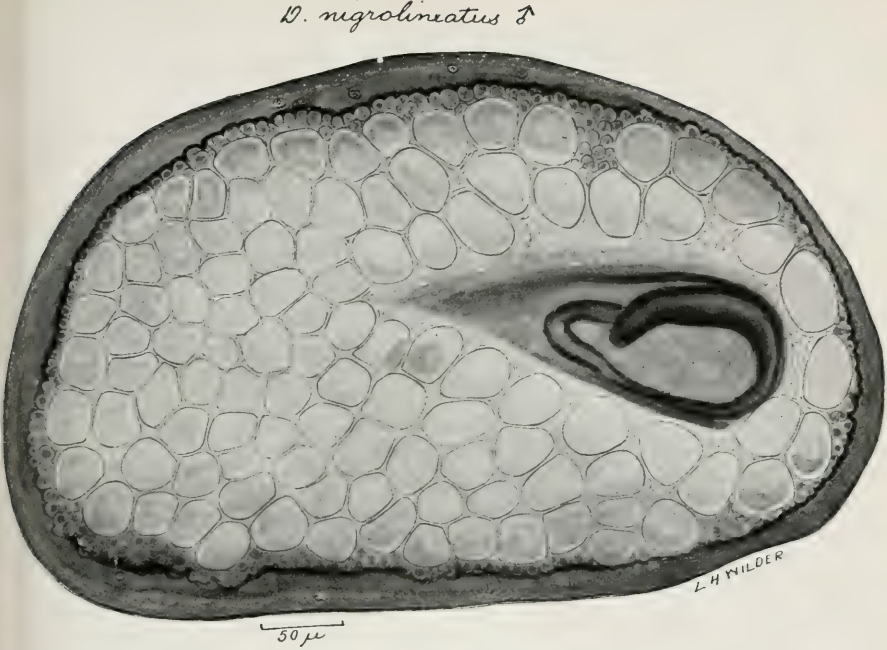


FIG. 22.—Stigmal plate of male *D. nigrolineatus* from New York. Enlarged. Original. U. S. P. H. & M. H. S. 9992.

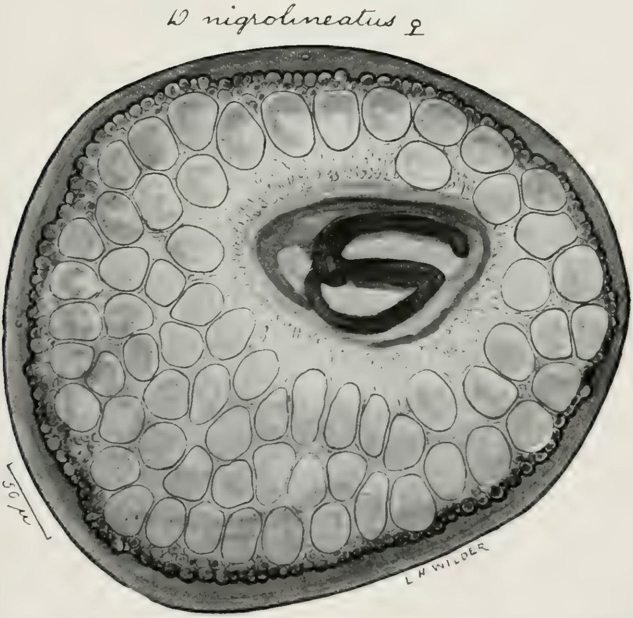


FIG. 23.—Stigmal plate of female *D. nigrolineatus* from New York. Enlarged. Original. U. S. P. H. & M. H. S. 9992.

D nigrolineatus ♀

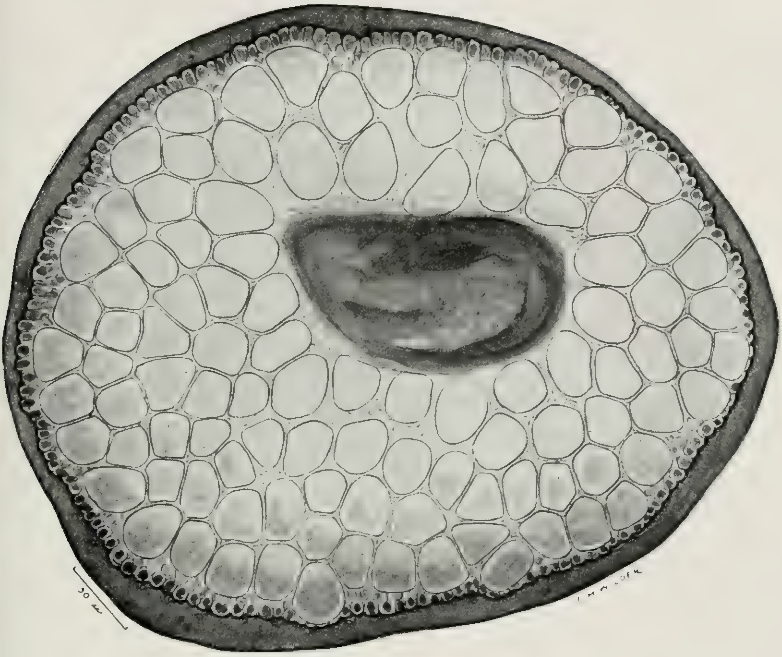


FIG. 24.—Stigmal plate of female *D. nigrolineatus* from Tennessee. Enlarged. Original. B. A. I. 4288.

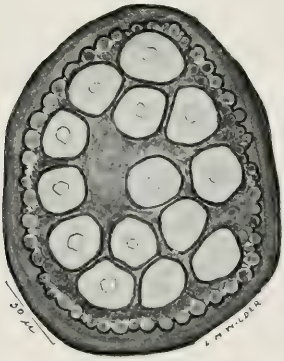


FIG. 25.—Stigmal plate of nymph *D. nigrolineatus* from Oklahoma. Enlarged. Original. B. A. I. 4363.

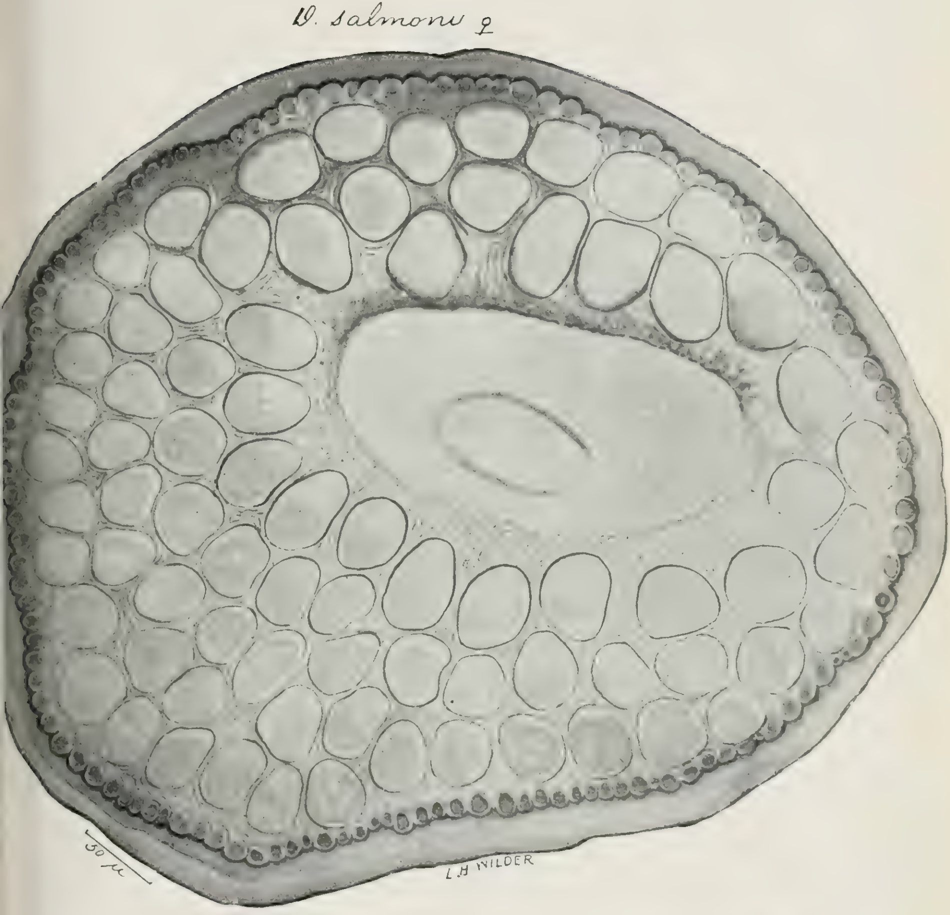


FIG. 26.—Stigmal plate of female *D. salmoni* (type) from *Bos taurus*, Oklahoma. Enlarged. Original. B. A. I. 3179.

D salmoni ♀

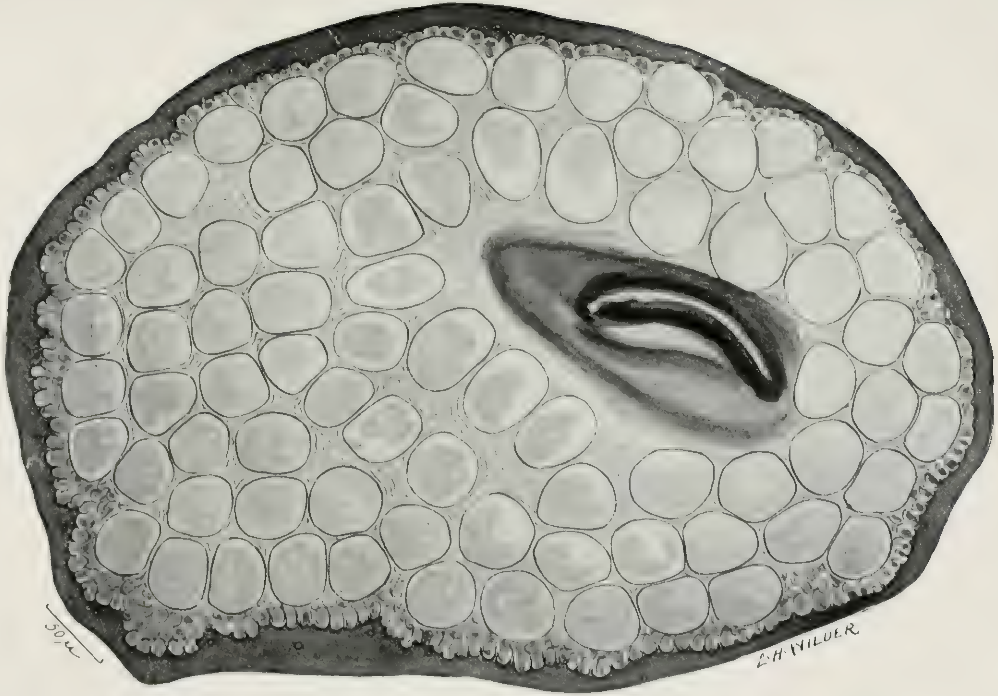
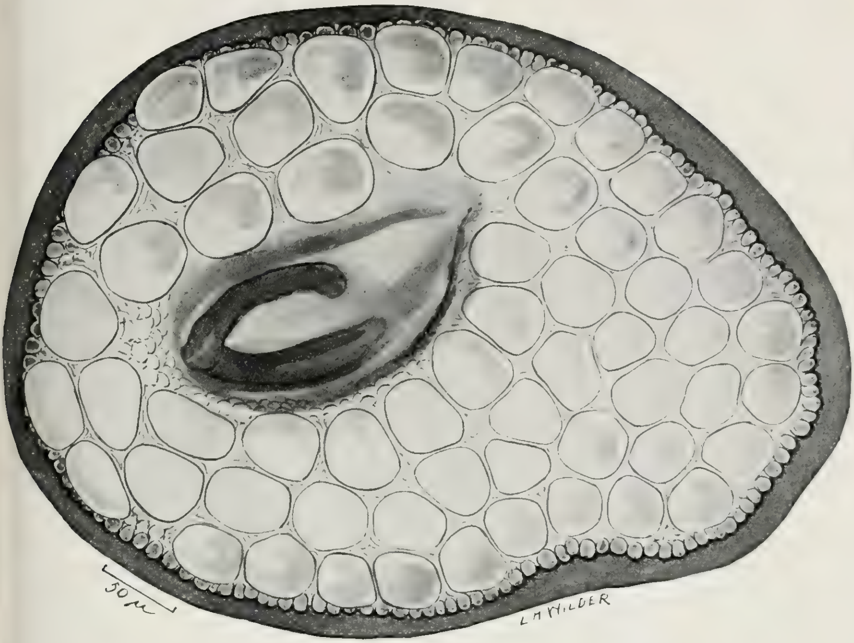
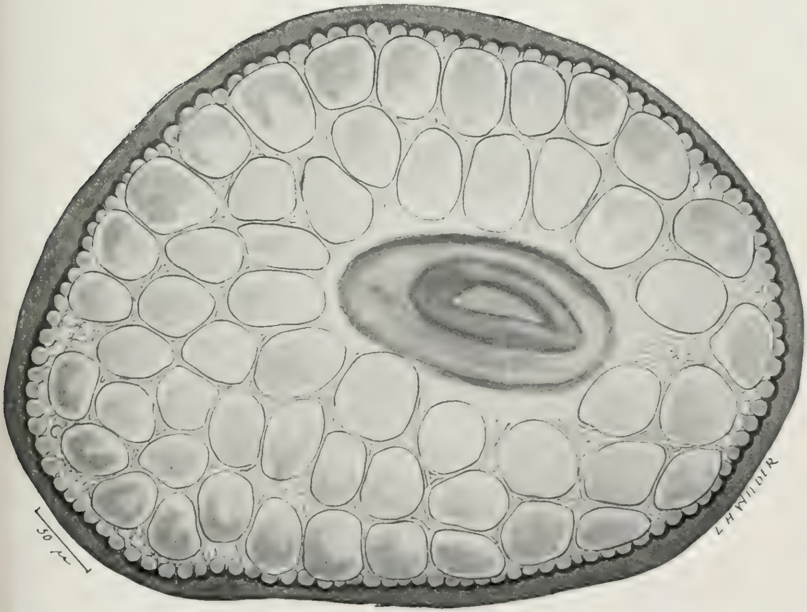


FIG. 27. Stigmal plate of female *D. salmoni*. Enlarged. Original. B. A. I. 2998.

D. salmoni ♂



D salmoni ♂



FIGS. 28–29. A pair of stigmal plates of male *D. salmoni* from Montana. Enlarged. Original. U. S. P. H. & M. H. S. 9724.

D salmoni ♀

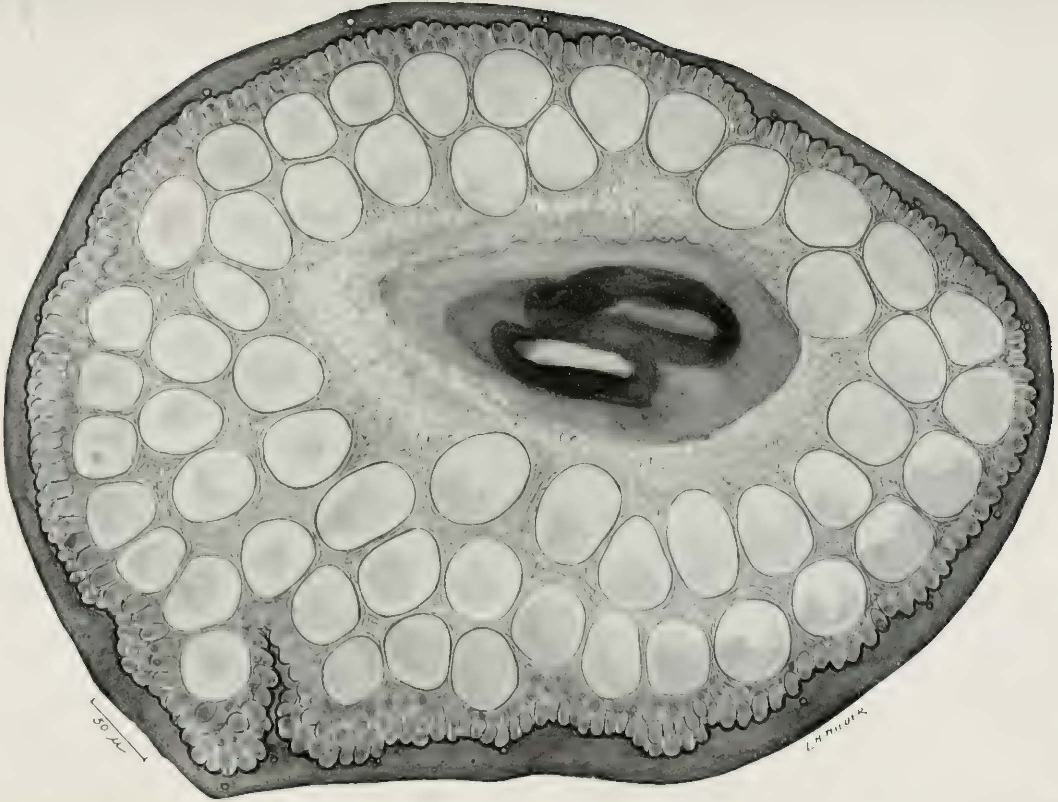


FIG. 30-31.—A pair of stigmal plates of female *D. salmoni* from Montana. Enlarged. Original. U. S. P. H. & M. H. S. 9724.

D salmoni ♀

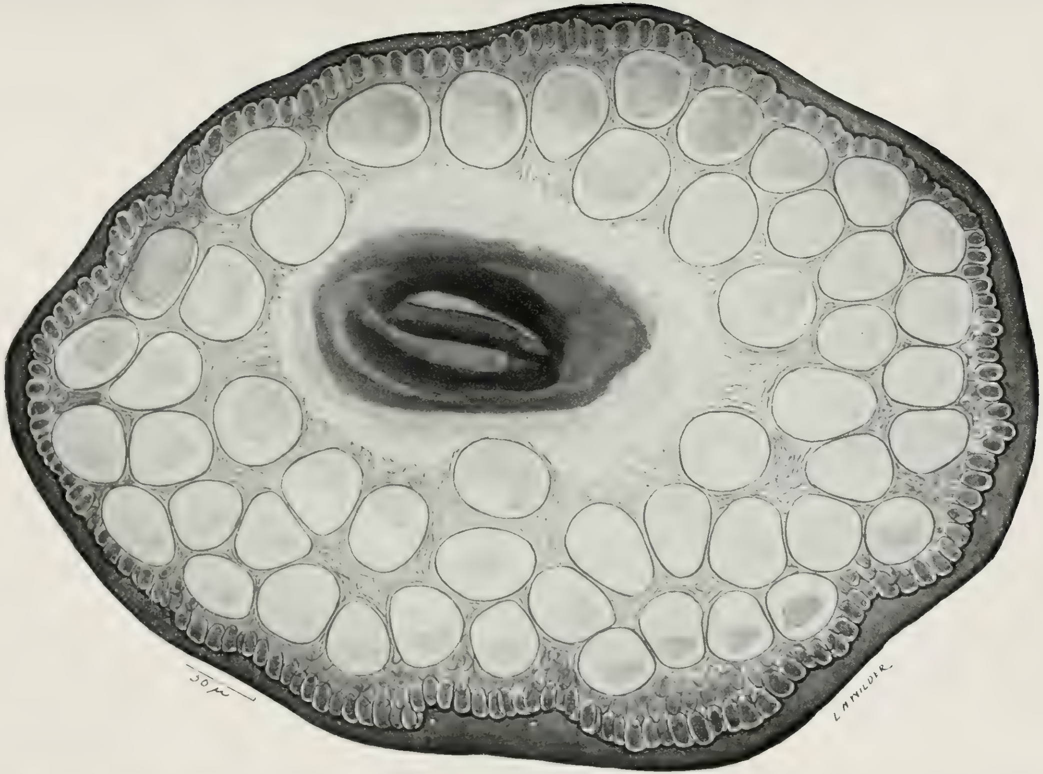


Fig. 30-31.—A pair of stigmal plates of female *D. salmoni* from Montana. Enlarged. Original. U. S. P. H. & M. H. S. 9724.

D salmoni ♀

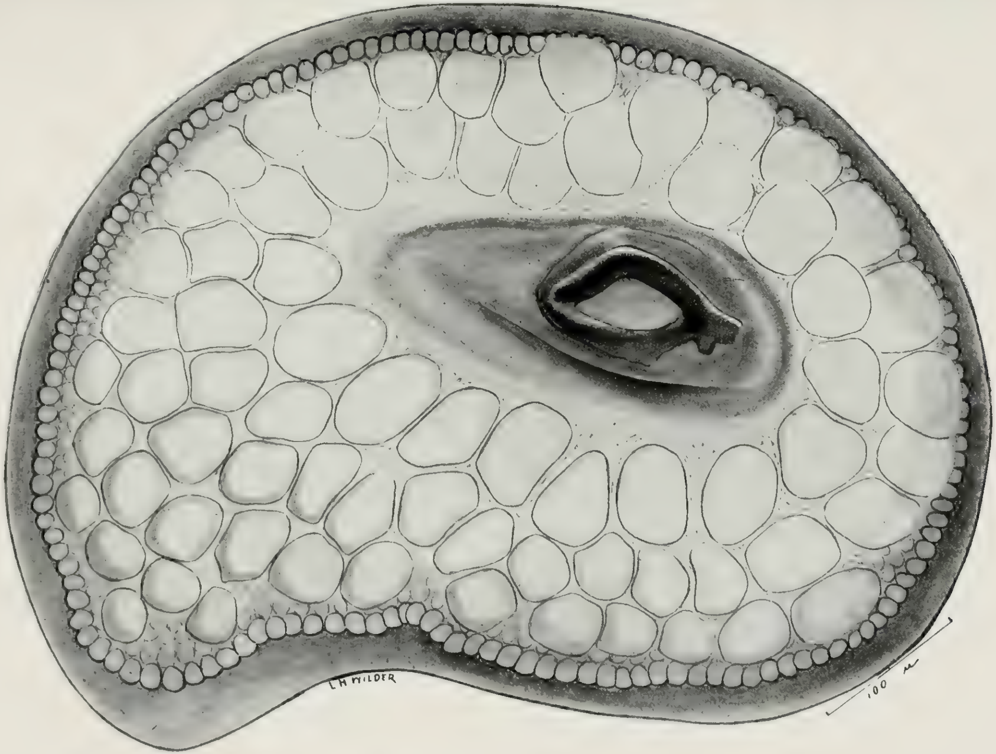


FIG. 32.—Stigmal plate of female *D. salmoni* from Tennessee. Enlarged. Original. B. A. I. 3206.

D. salmoni ♀

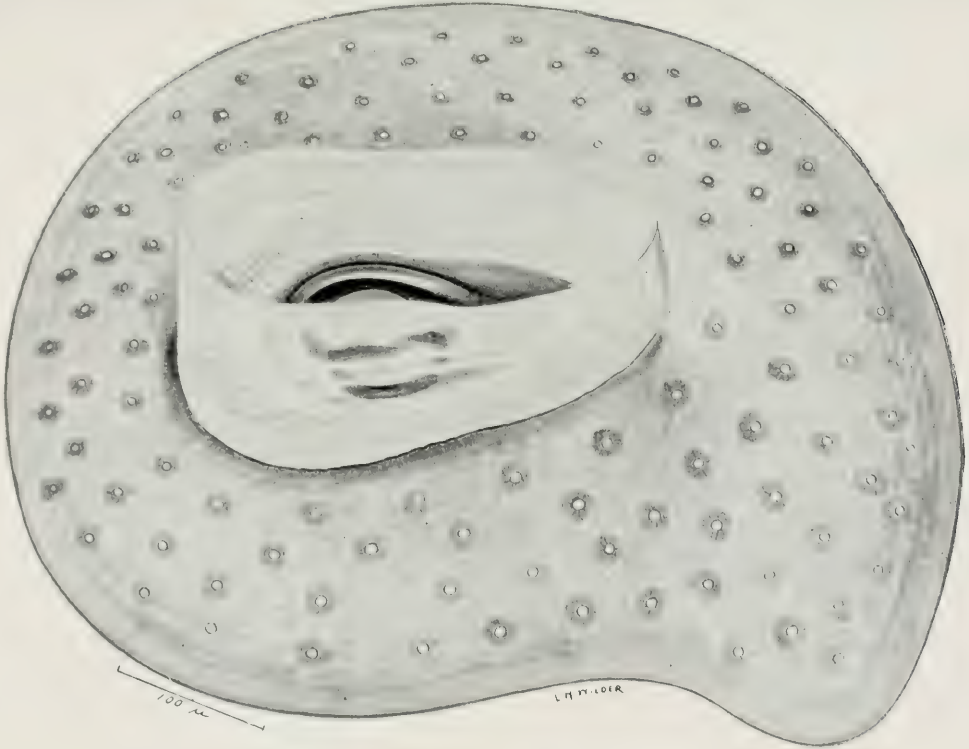
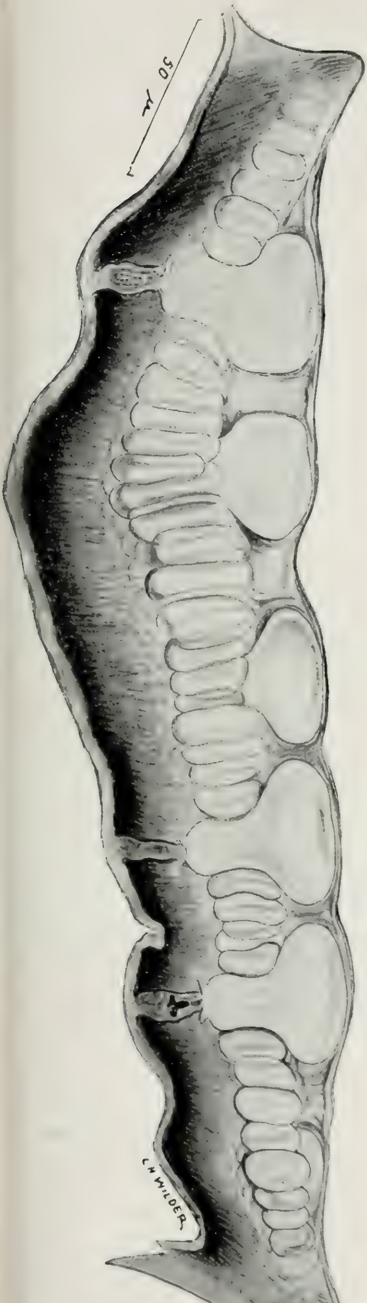


FIG. 33.—Obverse view of plate shown in figure 32.



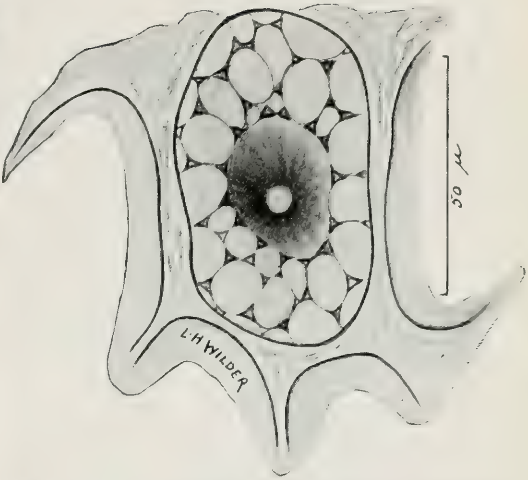


FIG. 34b.—View of a goblet of *D. salmoni*, at deep focus to show the supporting cells and the stem of the goblet. Greatly enlarged. Original. B. A. I. 3206.

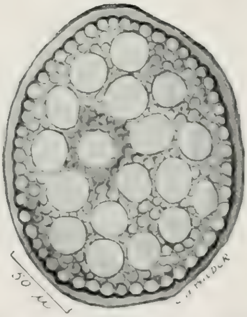


FIG. 35.—Stigmal plate of nymph of *D. salmoni*. Enlarged. Original. B. A. I. 3206.

D salmoni ♂

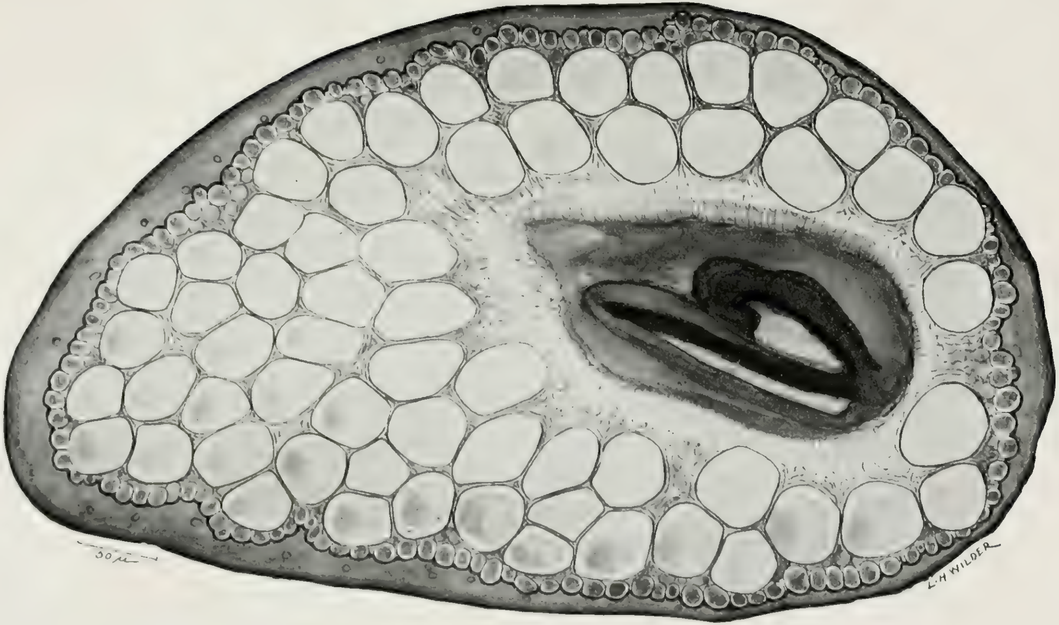


FIG. 36.—Stigmal plate of male *D. salmoni*. Enlarged. Original. U. S. P. H. & M. H. S. 10003.

D. variegatus ♂

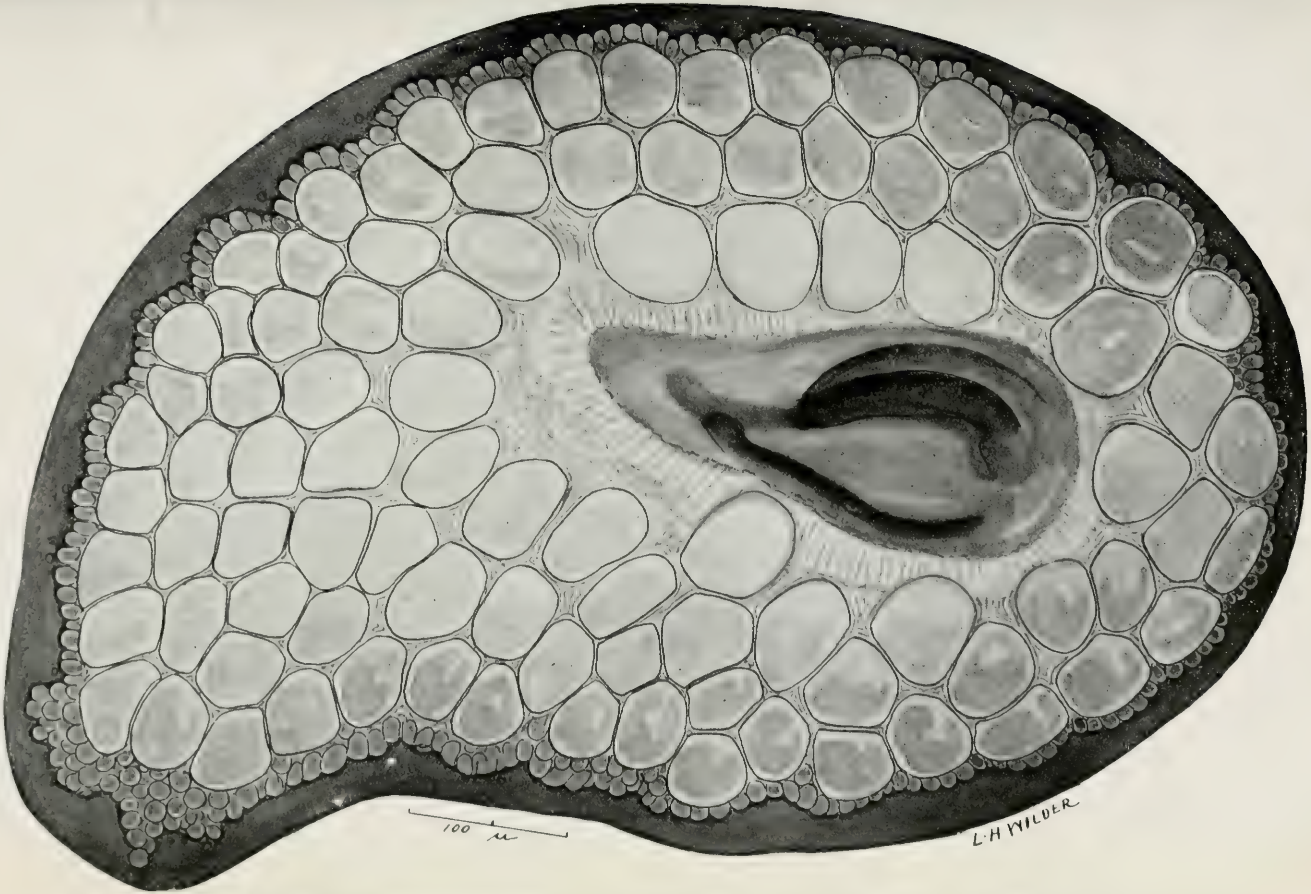


Fig. 37.—Stigmal plate of male *D. albipictus* seu *variegatus*. Notice the prolongation, and the large goblets. Enlarged. Original. B. A. I. 3172.

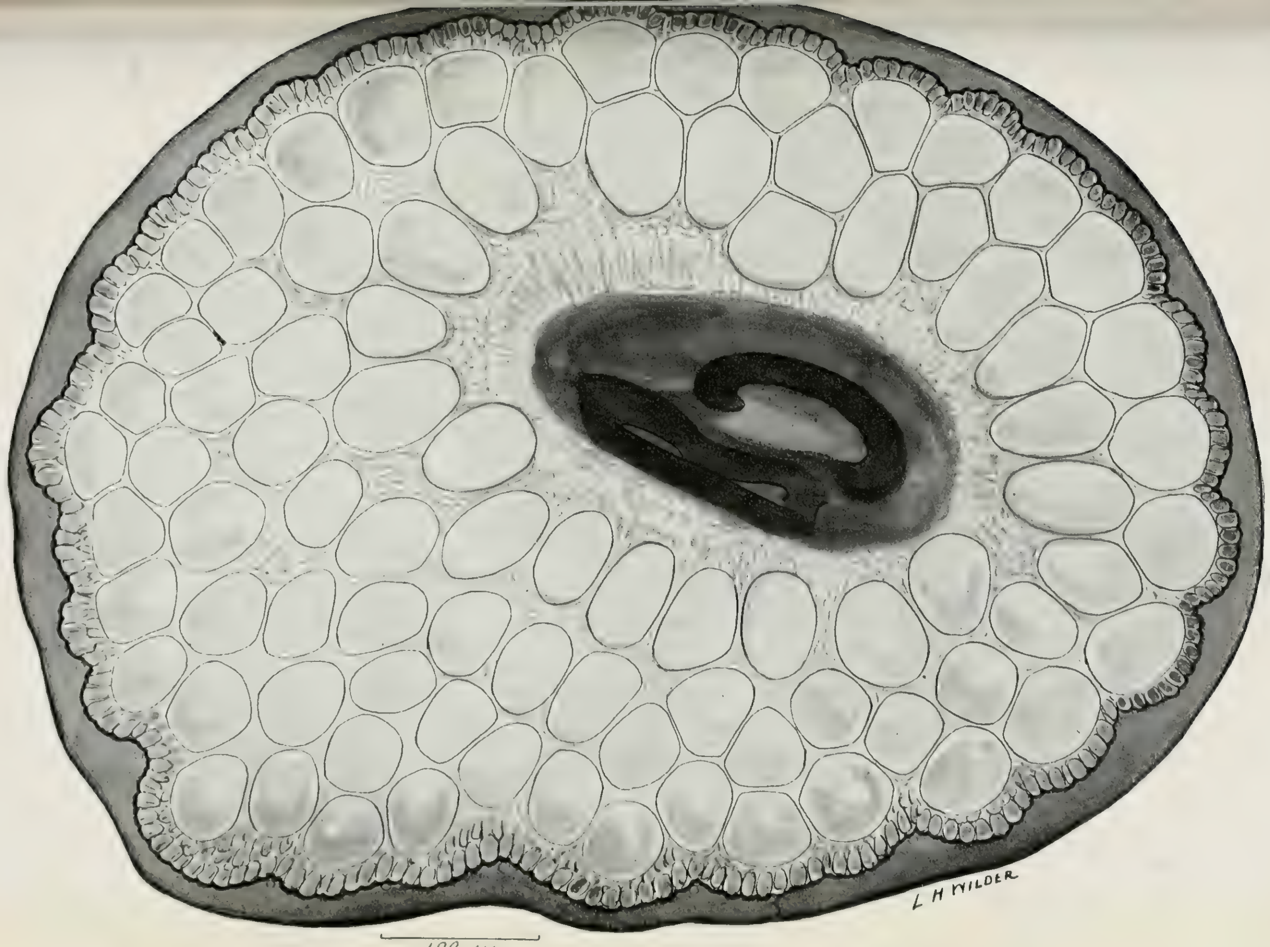


Fig. 38.—Stigmal plate of female *D. albipictus* seu *variegatus*. Enlarged. Original. B. A. I. 3172.

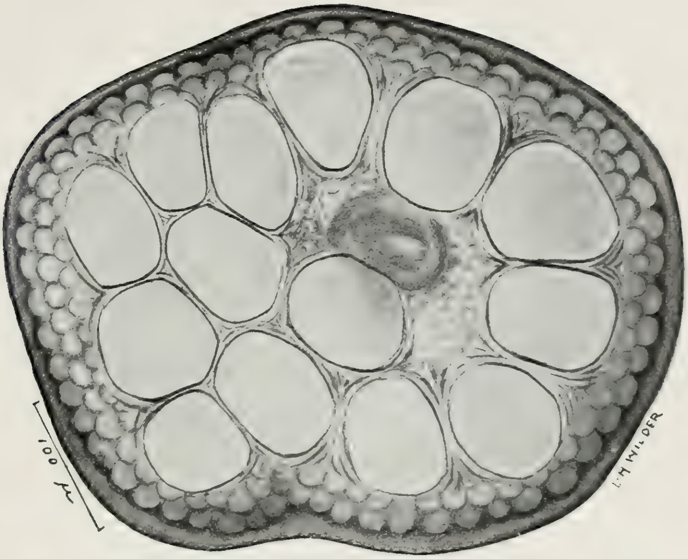


FIG. 39.—Stigmal plate of nymph *D. albipictus* seu *variegatus*. Enlarged. Original. B. A. I. 3172.

D nitens ♂

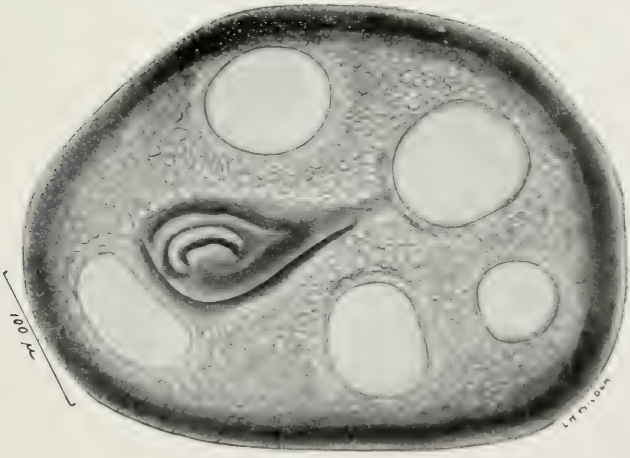


FIG. 40.—Stigmal plate of male *D. nitens* from Texas. Enlarged. Original. U. S. P. H. & M. H. S. 9908.

D nitens ♂

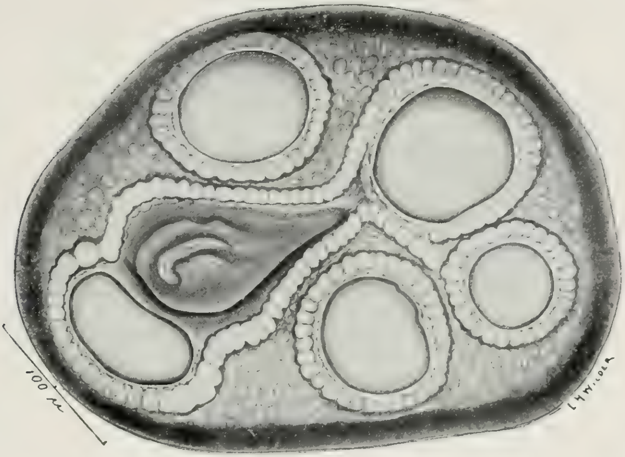
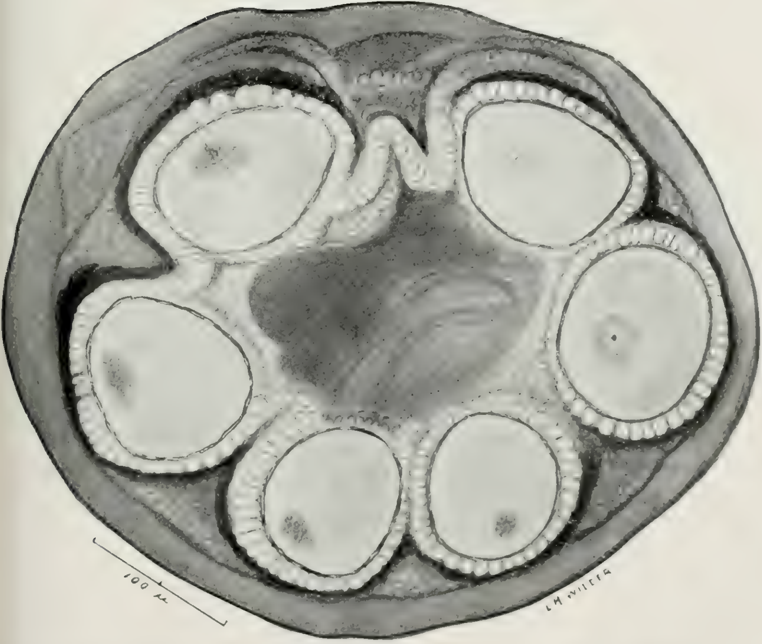


FIG. 41.—The same at lower focus.

D nitens ♀



. 42.—Stigmal plate of female *D. nitens* from Jamaica. Enlarged. Original. U. S. P. H. & M. H. S. 9991.

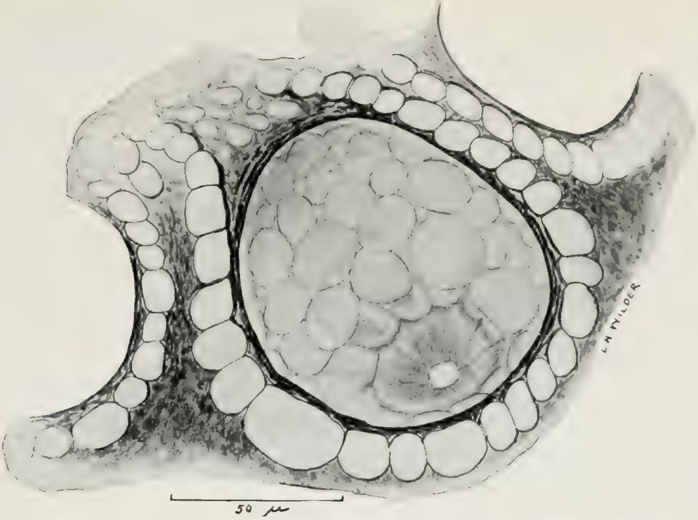


FIG. 43.—A goblet very greatly enlarged, showing also the supporting cells and the stem of the goblet. Greatly enlarged. Original. U. S. P. H. & M. H. S. 9908

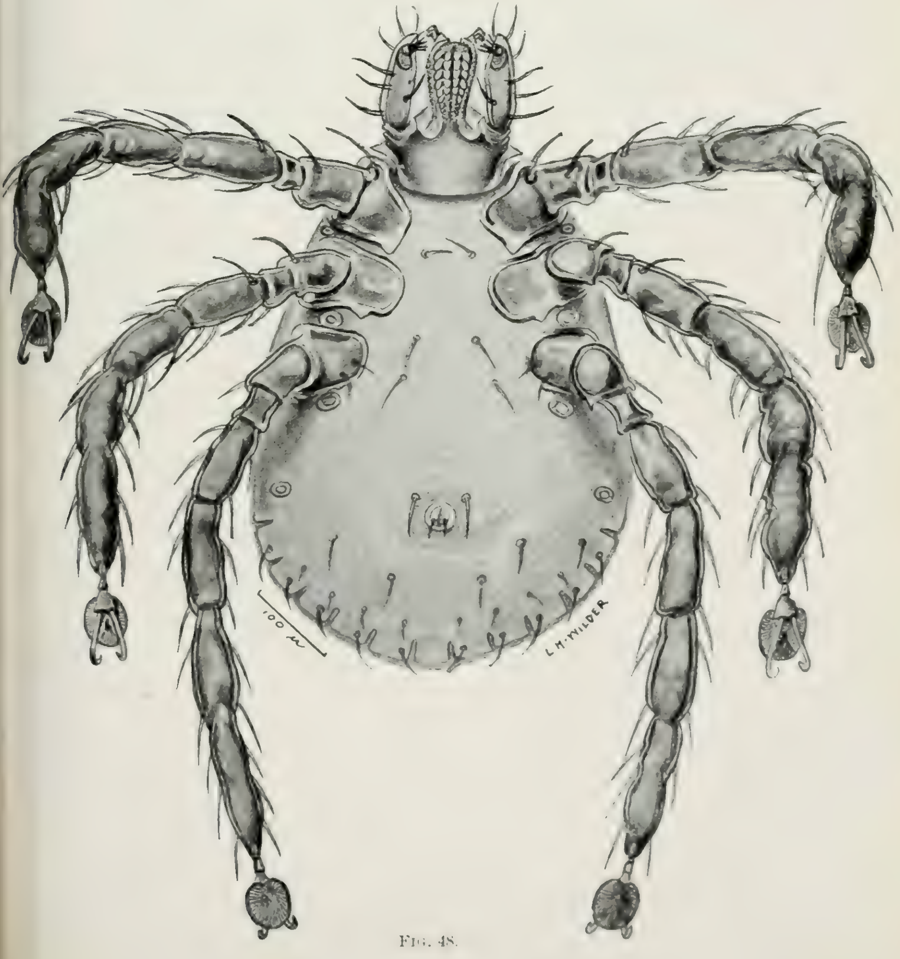


FIG. 48.

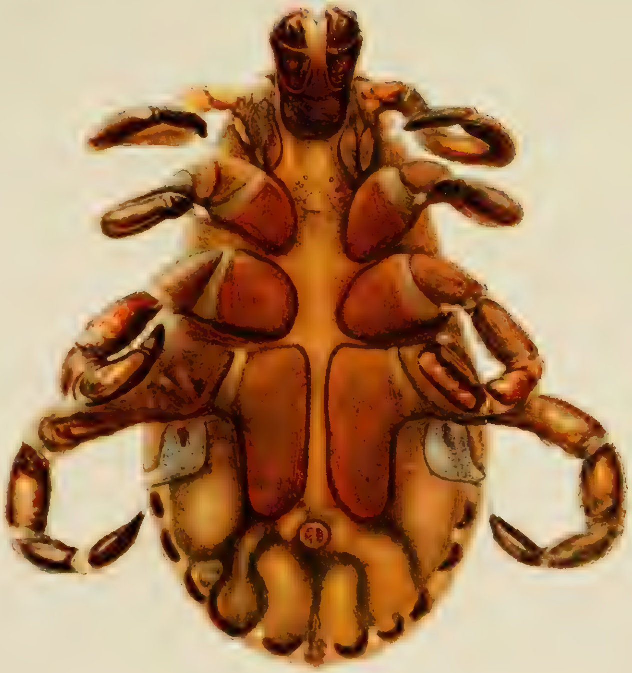


FIG. 44. VENTRAL VIEW.

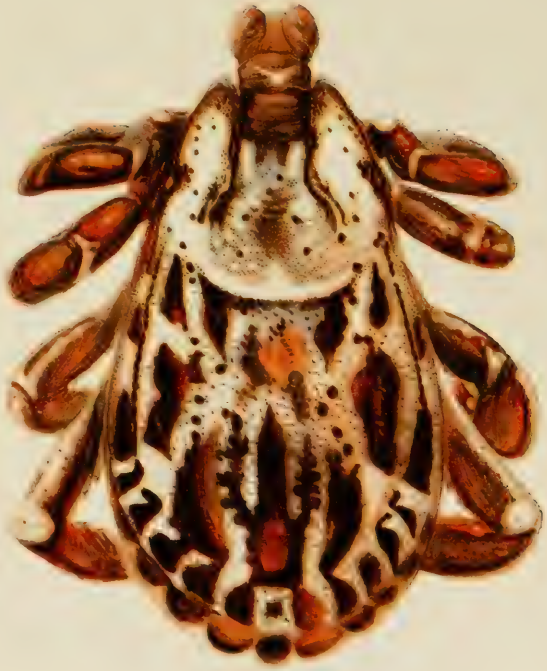


FIG. 45. DORSAL VIEW

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DERMACENTOR ANDERSONI.

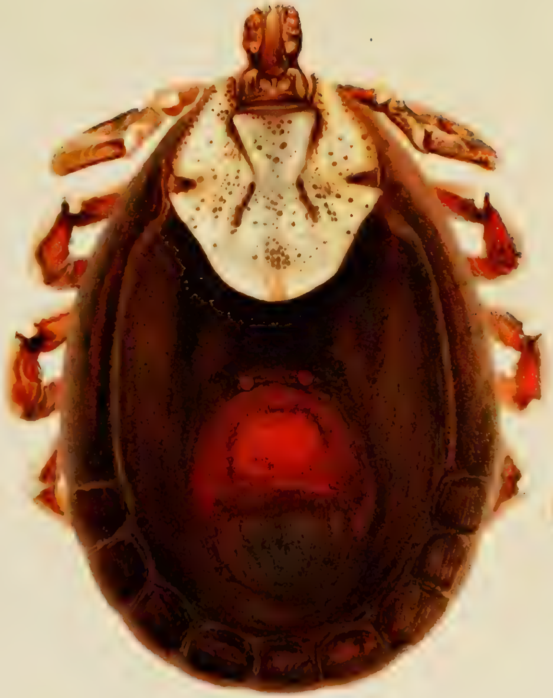

FIG. 46. DORSAL VIEW.

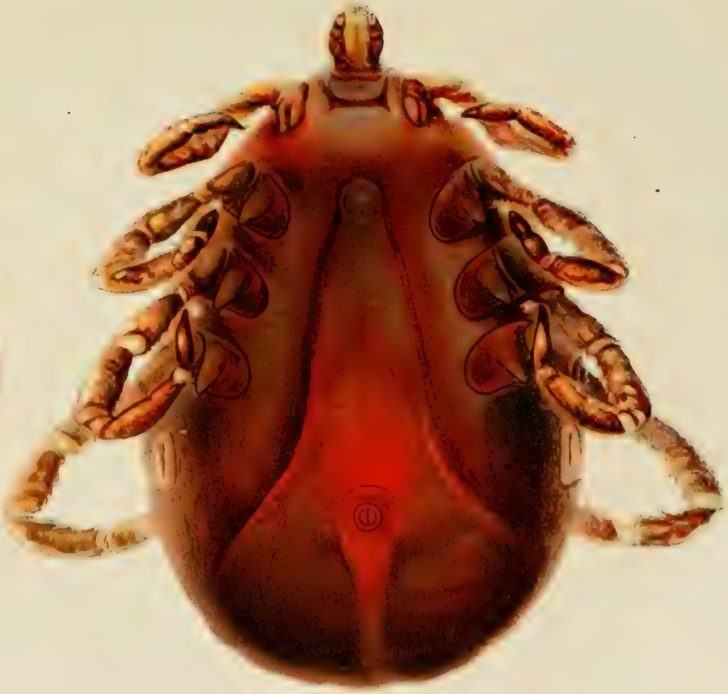

FIG. 47. VENTRAL VIEW

L. H. Wilder

DERMACENTOR ANDERSONI.

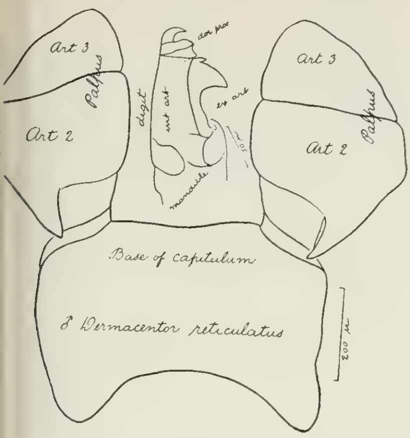

Fig. 49.

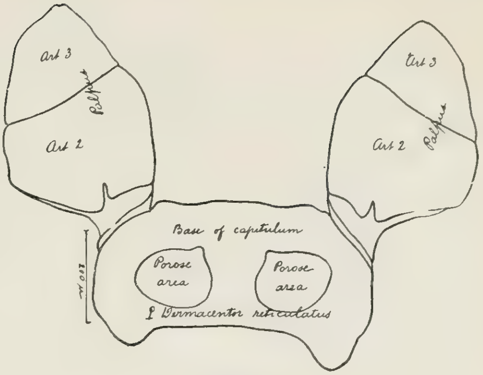

Fig. 50.

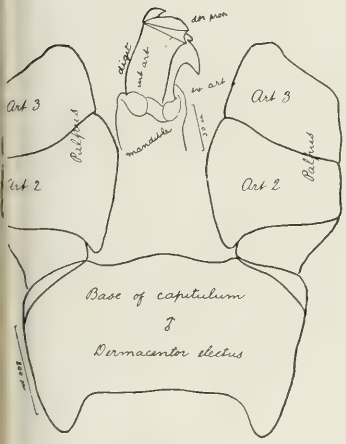

Fig. 51.

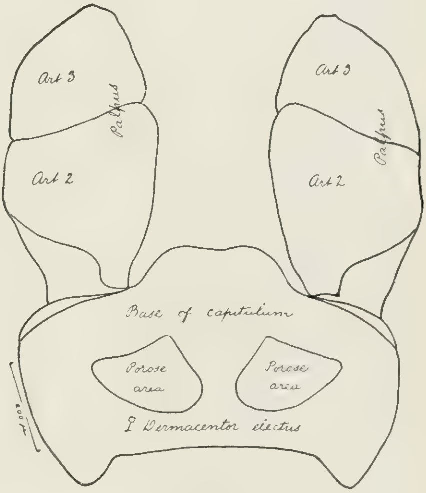

Fig. 52.

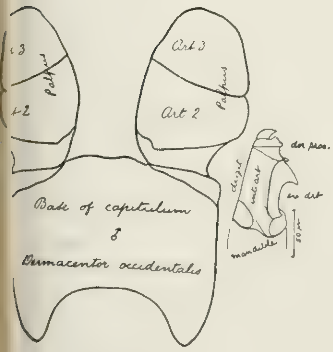

Fig. 53.

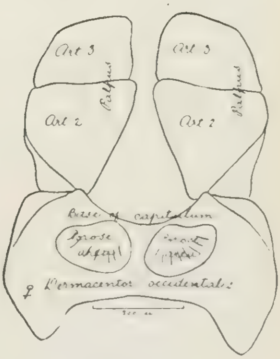

Fig. 54.

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Figs. 49-54.—OUTLINES OF CAPITULUM AND DIGIT OF DERMACENTOR

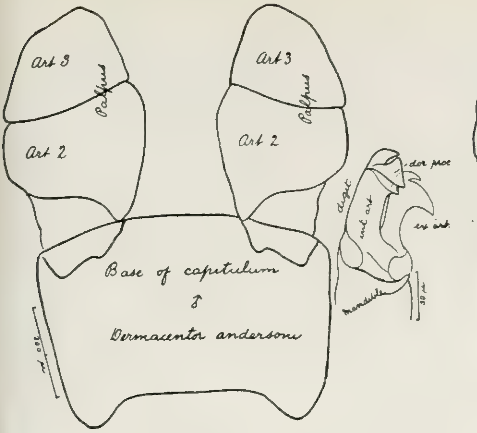

Fig. 55.

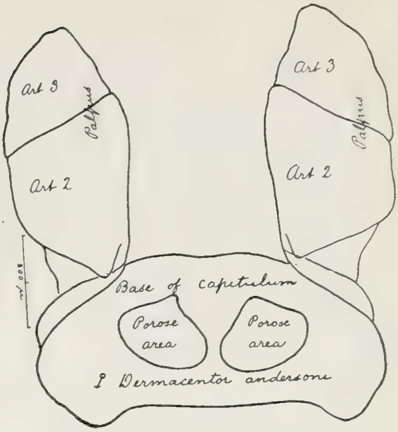

Fig. 56.

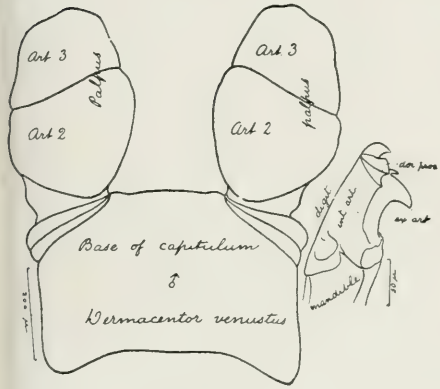

Fig. 57.

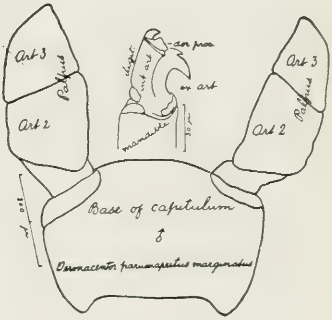

Fig. 58.

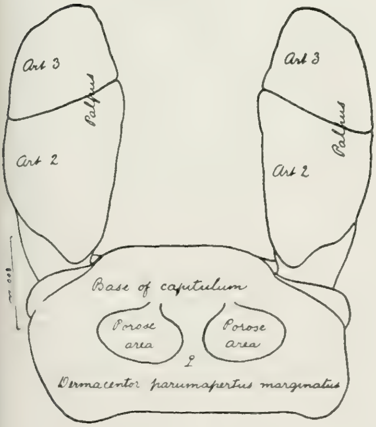

Fig. 59.

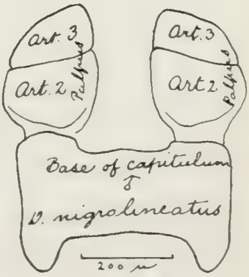

Fig. 60.

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Figs. 55–60.—OUTLINES OF CAPITULUM AND DIGIT OF DERMACENTOR.

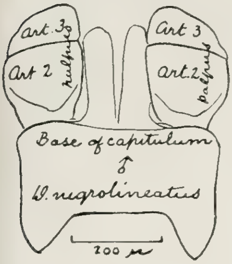

Fig. 61.

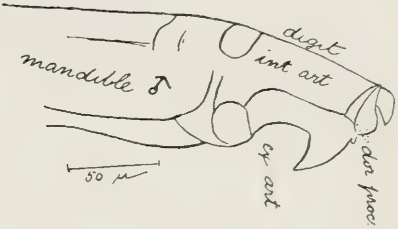

Fig. 62.

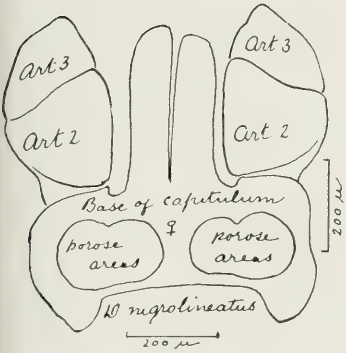

Fig. 63.

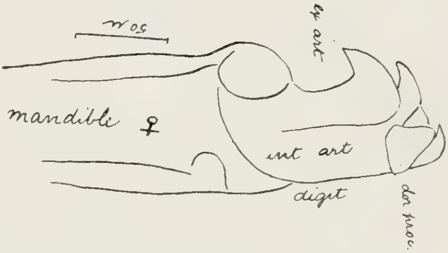

Fig. 64.

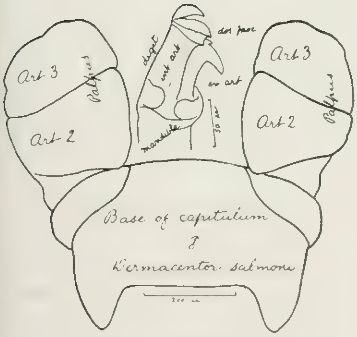

Fig. 65.

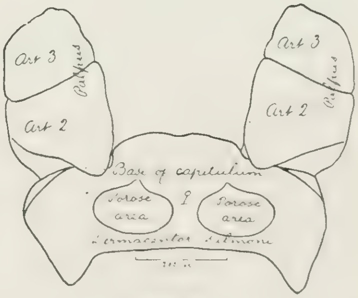

Fig. 66.

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Figs. 61–66.—OUTLINES OF CAPITULUM AND DIGIT OF DERMACENTOR.

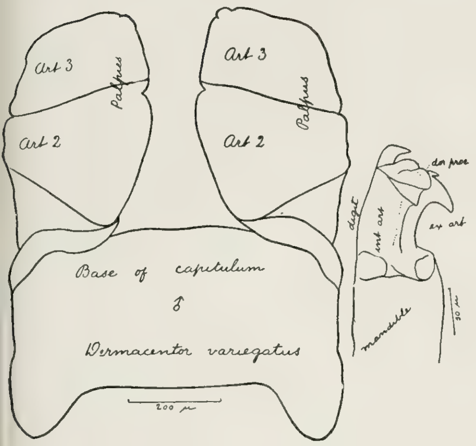

Fig. 67.

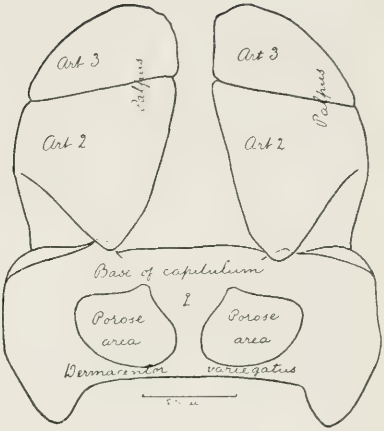

Fig. 68.

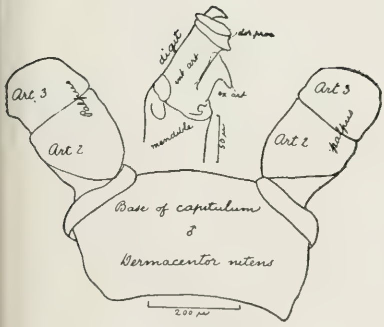

Fig. 69.

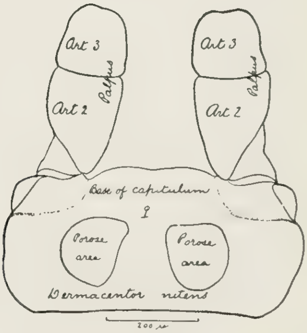

Fig. 70.

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Figs. 67–70.—OUTLINES OF CAPITULUM AND DIGIT OF DERMACENTOR.

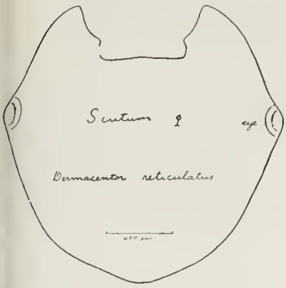


Fig. 71.

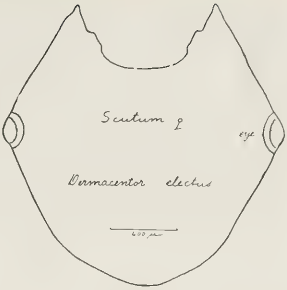


Fig. 72.

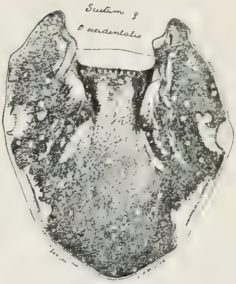


Fig. 73.

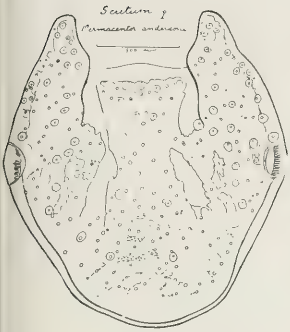


Fig. 74.

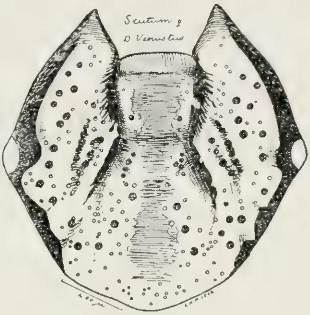


Fig. 75.

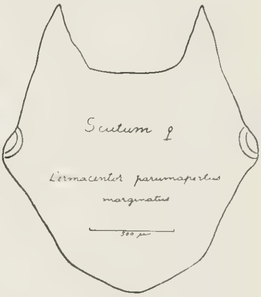


Fig. 76.

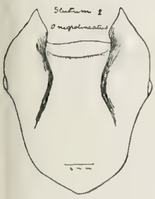


Fig. 77.

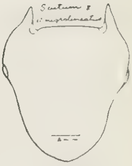
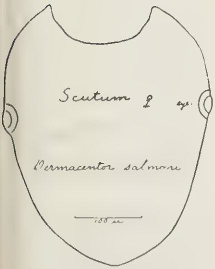


Fig. 78.



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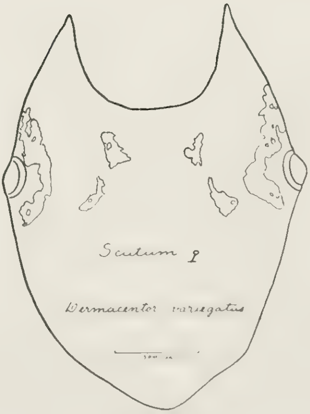

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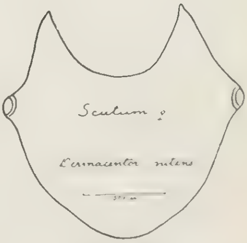

Fig. 81.

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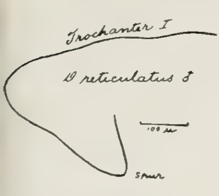
Figs. 71-81.—SCUTUM OF FEMALE OF DERMACENTOR



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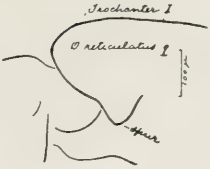

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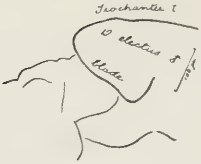

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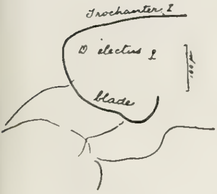

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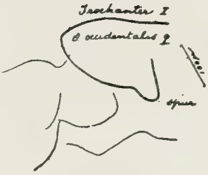

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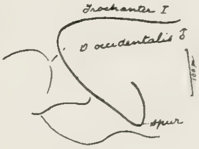

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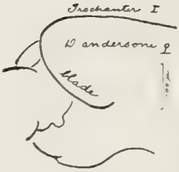

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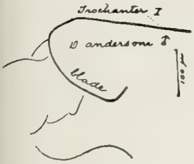

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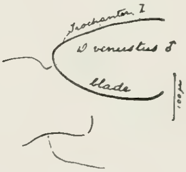

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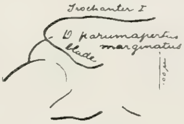

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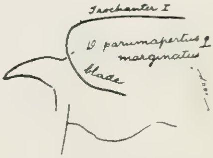

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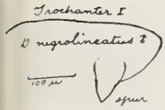

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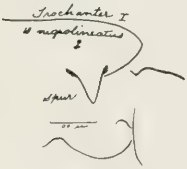

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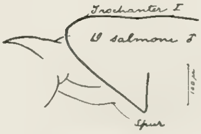

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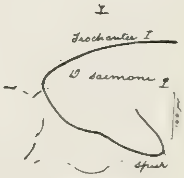

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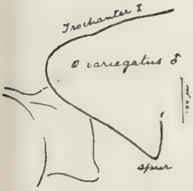

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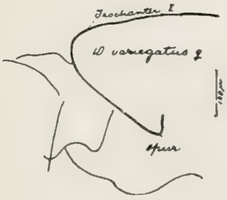

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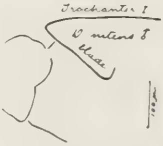

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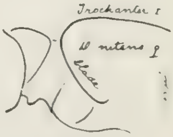

Fig. 100.

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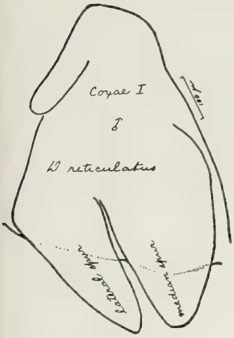
Figs. 82-100.—DORSAL VIEW OF TERMINAL PORTION OF TROCHANTER I OF DERMACENTOR



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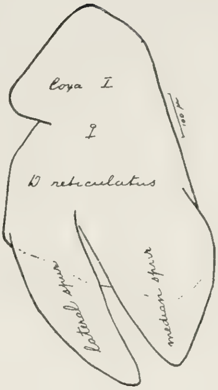

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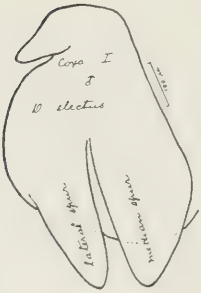

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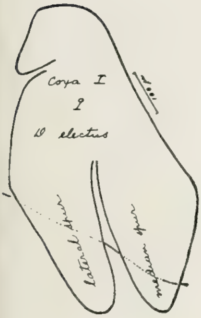

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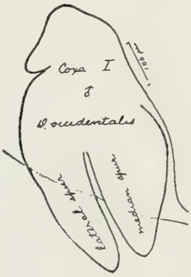

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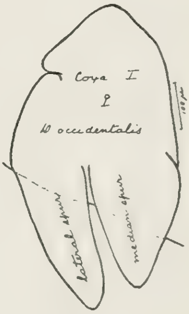

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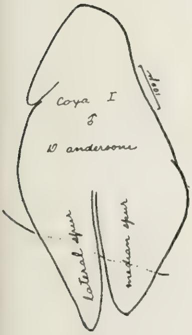

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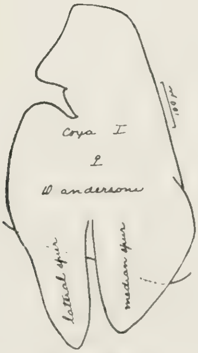

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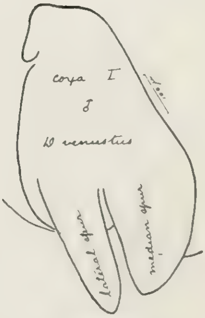

Fig. 109.

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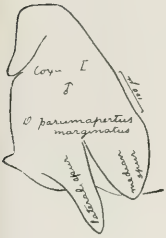
Figs. 101-109.—COXA I OF DERMACENTOR.



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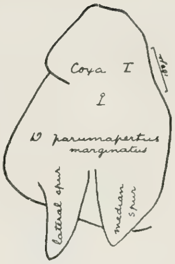

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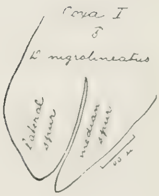

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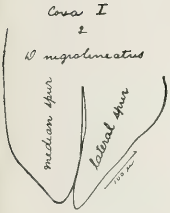

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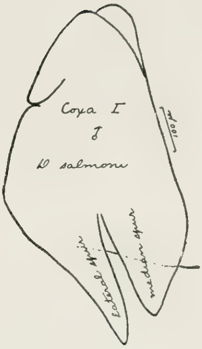

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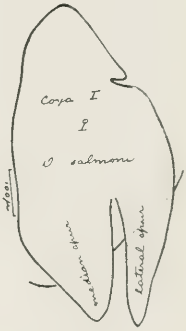

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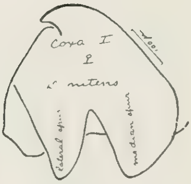

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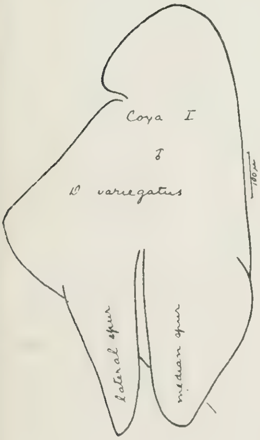

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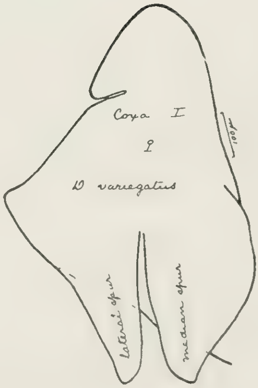

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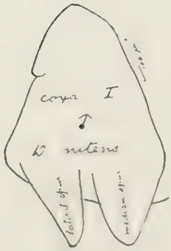

Fig. 119.

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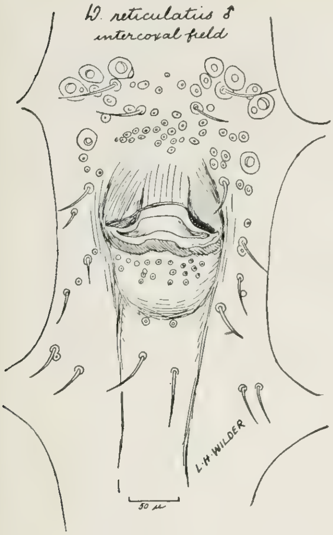
Figs. 110–119.—COXA I OF DERMACENTOR.



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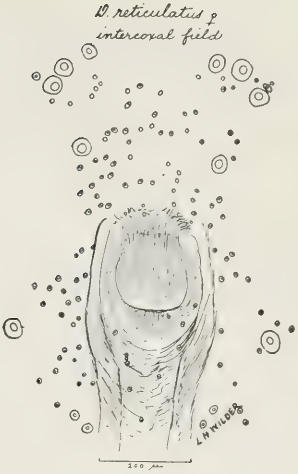

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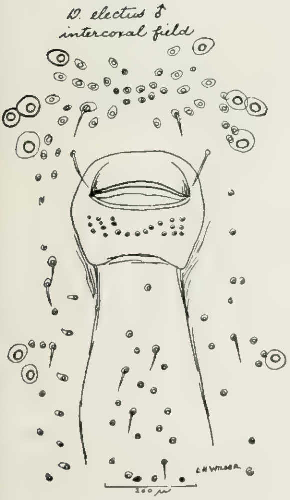

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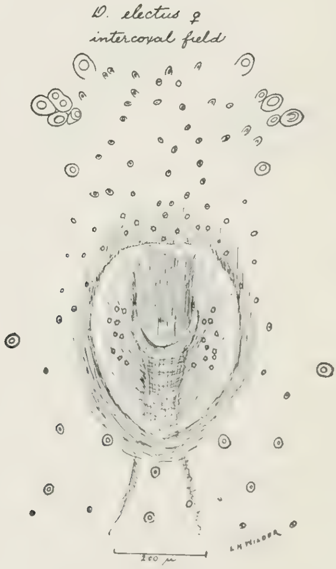

Fig. 123.

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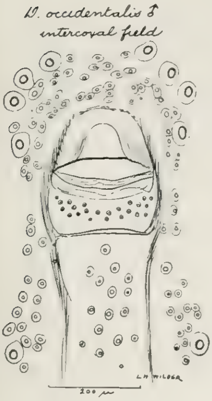
Figs. 120–123.—INTERCOXAL FIELDS OF DERMACENTOR.



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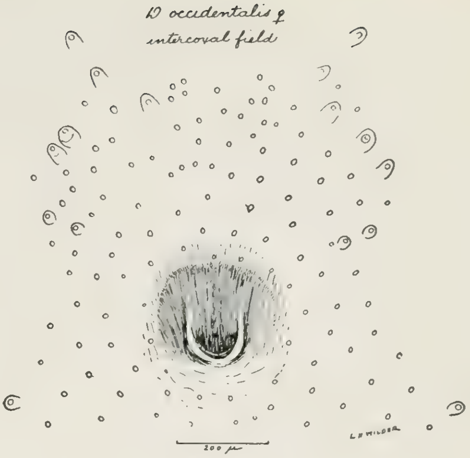

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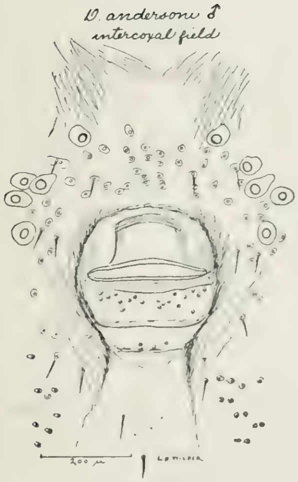

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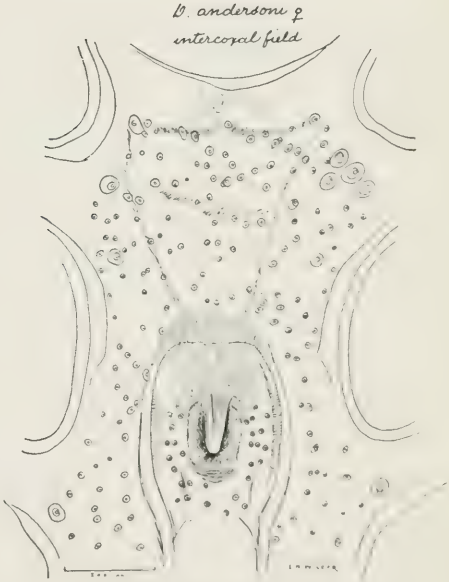

Fig. 127.

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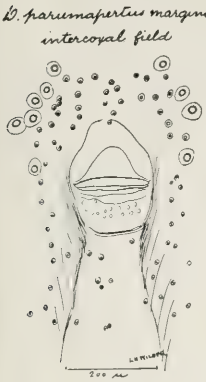
Figs. 124–127.—INTERCOXAL FIELDS OF DERMACENTOR.



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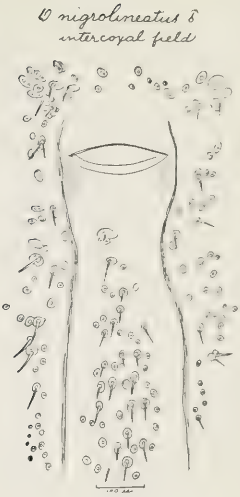

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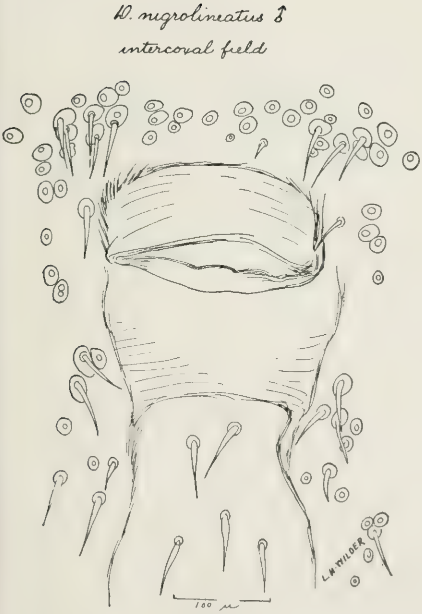

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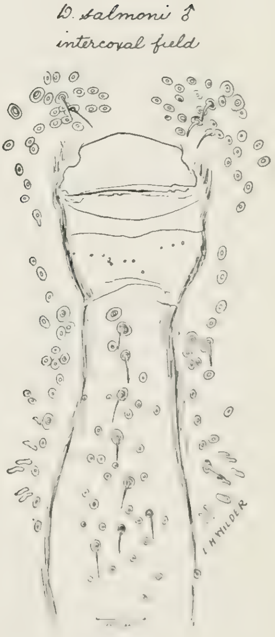

Fig. 131.

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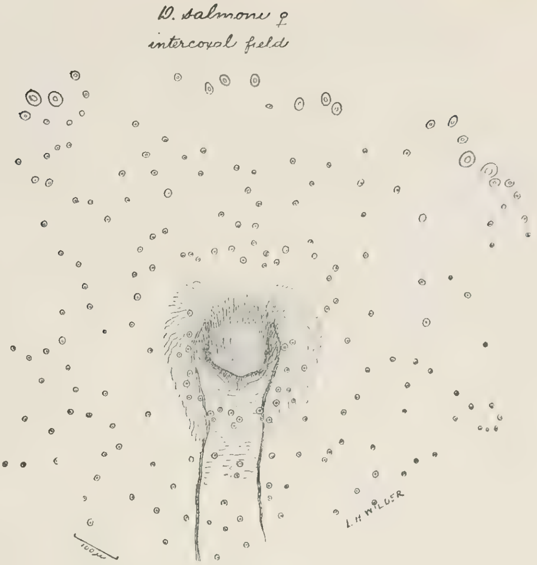
Figs. 128–131.—INTERCOXAL FIELDS OF DERMACENTOR.



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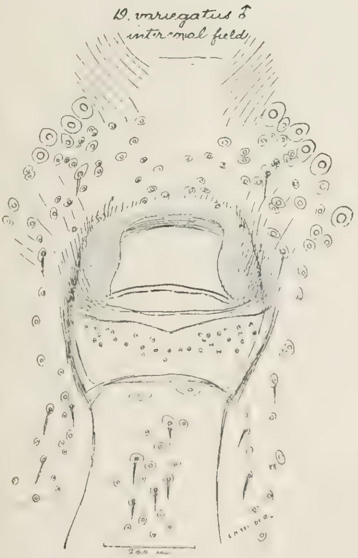

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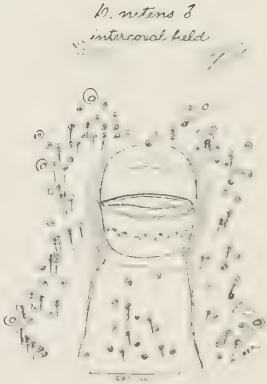

Fig. 134.

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Figs. 132-134.—INTERCOXAL FIELDS OF DERMACENTOR.

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